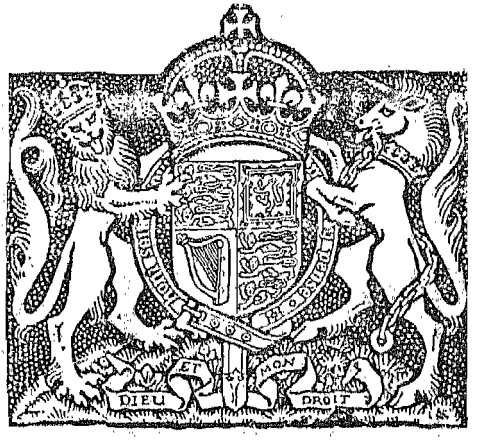

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THE INTELLIGENT USE OF THE MICROSCOPE

THE INTELLIGENT USE OF THE MICROSCOPE

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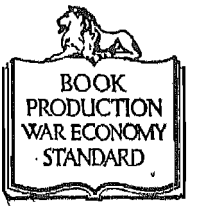

THIS BOOK IS PRODUCED IN COMPLETE
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PREFACE

My father gave me my first microscope when I was seven years of age. It is a tribute to my father's intelligence that this was not the usual toy, but a very sound instrument, though it was equipped with a composite lens. I do not claim, however, to be an expert on the microscope, though I have handled a great many since that early start; but I do claim that the non-expert is often better qualified to write a handbook for beginners. Their problems, queries, and difficulties are more intelligible to him. I would have been saved a truly enormous amount of unnecessary labour, misgivings, and uncertainties if I could have found a handbook combining thoroughness with order and simplicity. My attempts to do so have filled several shelves in my library with books, many admirable, and some futile, but no one of which really contained just what was wanted, all that was wanted, and nothing but what was wanted. This is the object I set out to achieve; whether I have succeeded or not, necessarily depends on the reader's own way of viewing things, but even if this handbook proves useful to only a few students, I shall consider myself well satisfied.

My thanks are due, and gratefully given, to all the manufacturers mentioned for their generous help and advice, and in particular, I would like to thank my fellow members of the Royal Microscopical Society, and the Association for Scientific Photography for their assistance.

C. W. OLLIVER, A.M.I.E.E., F.R.M.S.

London, *28th August*, 1946

I dedicate
THIS BOOK
to my mother-in-law, who
gave me my first perfect
instrument at a time when
I sorely needed it.

CONTENTS

"Nothing has such power to broaden the mind as to investigate systematically and truly all that comes under thy observation in life."

MARCUS AURELIUS

CHAPTER I

INTRODUCTION

THERE are many books on this subject. Some are intended for the amateur, some for the student, and some even for the expert. Acquiring knowledge is largely a matter of proportion and relationship. It is useful to be able to refer new facts to a simple and orderly framework within which they fit easily and naturally into their proper places.

This book is intended to provide such a framework. It is comprehensive only inasmuch as it contains a complete outline with the essential features sketched in.

Such outlines are given to children when they are learning to draw. Later, they will learn how to draw the outlines for themselves, but in the meantime they will have been saved much perplexity, and will produce quite convincing pictures.

The child, however, may never take the trouble to learn. The student, looking down a microscope, will find that he can apparently obtain a satisfactory image though he has neglected all but the most elementary rules. A careless photographer often gets a good negative. He just as often gets inexplicable failures. So will the student and he will fail, like the photographer, when he first encounters a difficult and correspondingly important subject.

The occasional holiday photographer does not use a Leica any more than the professional uses a Brownie, yet both give satisfactory results within their own particular fields.

Similarly, the microscope is often treated as an ideal optical instrument, whereas it is really an instrument with several degrees of efficiency.

A lower degree of efficiency is sufficient for routine work, and even for many forms of research involving objects which are considerably above the limits of microscopic vision.

The highest degree of efficiency is only required for work approaching the limits of resolution, and for photomicrography.

This is important because the beginner is easily discouraged unless he realises that the delicate adjustments required for critical work are not only tedious, but wasteful and unnecessary when rigidly applied to low power work or routine examinations.

Considerable latitude is permissible. An experienced microscopist is one who knows the rules which can, and those which cannot, be ignored according to circumstances.

This is what is meant by the intelligent use of the microscope.

It is essential for the beginner to experiment with the various methods described, strictly observing the rules. He will soon be able to appreciate the difference between a critical image, and one that is not. Then, and only then, will he be in a position to judge how far he may depart from the rules with safety, while remaining within the limits of correct microscopic vision.

There are two extremes which I believe should be avoided: too much theory, divorced from its practical implications, and the rule-of-thumb type of instruction which remains unexplained. One should not separate the hows from the whys.

It is impossible to do a thing well unless one does it intelligently or, in other words, unless one knows just why it is being done. On the other hand, the reader's immediate object is the proper use of the microscope for a specific purpose, and he is naturally anxious to get to work. For that very reason he is apt to find rules tiresome and hence, to question their necessity. Just enough elementary theory is included in this book to make this plain, and no more.

The microscope, in its modern form, would appear to leave little to the skill of the operator. This is not really true. The two essential factors are the choice of the right object glass, and correct illumination. A good deal of space is devoted to these subjects which include magnification, numerical aperture, resolution, and methods of illumination.

Unlike many books of even more modest size, this one deals neither with mounting nor staining. A chapter on mounting thrown in for good measure is not only useless, but extremely misleading. The idea appears to be that the student need only purchase one book. This is an absurdity.

Preparing objects for examination under the microscope is a technique in itself, and it cannot be boiled down to a few useful hints, recipes, and formulae. A book of this size professing to deal with the use of the microscope as an instrument and the preparation of slides as an accessory would fall into a common error and achieve nothing useful.

There are no short cuts to the preparation of really satisfactory permanent mounts, nor is there any method which can be applied

with equal success to any object, or even to any group of objects. Patient study and experience are the first essentials, and the student in any particular field will soon find himself experimenting with new materials and evolving a technique of his own.

There are many excellent and comprehensive books dealing with mounting exclusively, and a glance through their pages will show the futility of the usual chapter on mounting included in elementary text-books.

Photomicrography, on the other hand, has become an integral part of the microscopist's work, and while it requires considerable skill and experience, yet it is governed by the same general rules as prevail for visual work. It is almost entirely a matter of correct illumination if this is taken as including the use of suitable filters.

Here again there are degrees of efficiency. A series of photographs may be rapidly taken to record successive phases of growth, for instance, but the accurate reproduction of minute detail may be of no importance. At other times a photograph may be required to show the finest detail. Obviously the same technique cannot efficiently be applied in both cases.

The intelligent use of the microscope therefore implies using the instrument with understanding; an understanding of its possibilities and limitations, of the principles of its design, and of the precise functions of its components and accessories.

This book is intended to be a guide to such understanding, that the student may not wander here and there, a prey to controversial theories and conflicting recommendations. As stated in the opening paragraphs of this introduction, it is an outline within which the student may fill in whatever detail he may require, drawing from the wealth of information contained in specialised periodicals, more advanced text-books, and manufacturers' catalogues.

CHAPTER II

OPTICAL THEORY

ELEMENTARY THEORY

Examination of Object with the Naked Eye

THE apparent size of an object, seen with the naked eye, depends on the angle subtended by the object at the optical centre of the eye. This angle is inversely proportional to the distance between the object and the eye. (This is not strictly true; the relationship is between the tangents of half the subtending angles.) Hence, the nearer an object is brought to the eye, the larger it will appear to be. At half the distance, it appears twice as

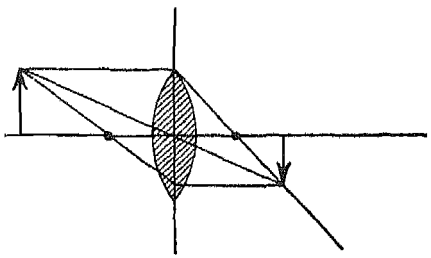


FIG. 1.—Real inverted image when object is outside principal focus of lens.

large, at a quarter of the distance, four times, etc. The simplest method of magnification is thus to bring the object closer to the eye.

The normal eye cannot, however, focus an object clearly if it is closer than the Minimum Distance of Distinct Vision, which is taken as being ten inches for a normal eye.

Use of Lens.—If we interpose a lens between the object and the eye, we assist the eye to focus an object placed at a much shorter distance. This is the principle on which the microscope is based. We shall then be looking, not at the object itself, but at an image formed by the lens.

Real and Virtual Images.—The following are the fundamental principles governing the formation of images with a simple lens:

(1) When an object is placed at a point *without* the principal focus of the lens, a *real inverted* image is formed on the opposite side of the lens. Such an image can be cast upon a screen, or photographed. (Fig. 1.)

(2) When an object is placed at a point *within* the principal focus of the lens, no real image is formed, but the eye, if placed in the emerging beam, will see a *virtual, erect,* and magnified image of the object on the same side of the lens as the object, and *apparently projected at the distance of distinct vision.* (Fig. 2.)

If the eye is now placed in the plane of the real image nothing will be seen but a confusing mass of light. But if the eye is withdrawn to a distance from the image approximately equal to

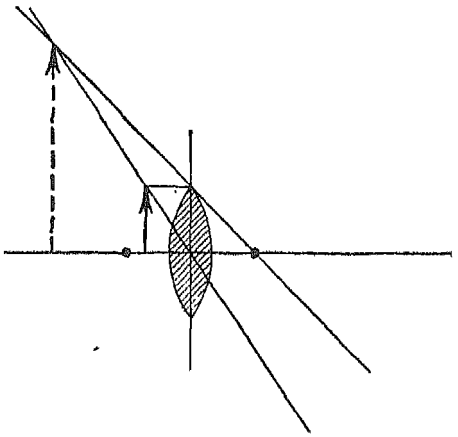


FIG. 2.—Virtual erect image formed when object is within principal focus of lens.

that of distinct vision, it will see an *inverted* image, suspended, so to speak, in mid-air. If, however, the object is *within* the focus of the lens, and the eye placed within the emerging beam, the virtual image will be readily seen. The image in this case is not a real one, and so would not form an image on a screen or photographic plate. It is also *erect.*

Magnification.—The normal distance of distinct vision is a reference distance against which the subtending angle of the virtual image can be projected and compared with that of the object itself when located ten inches from the eye.

Supposing we examine an object located at ten inches from the naked eye; when brought to a position one inch from the eye, the angle subtended, as already explained, will be ten times greater, but the object will be quite out of focus. In order to magnify the object ten times and obtain a sharply focused image, we will have to interpose a lens giving a virtual image of the object which

will subtend the same angle as the object subtends when placed one inch from the eye. This will be found to be a lens of one-inch focus.

It follows that the magnification will be given by the expression:

$$\text{Magnification} = \frac{\text{Distance of Distinct Vision}}{\text{Focal length of lens.}}$$

$$= \frac{10}{1} = 10$$

If the focal length of the lens is expressed in inches, the distance of distinct vision is taken as 10 inches. If the focal length of the

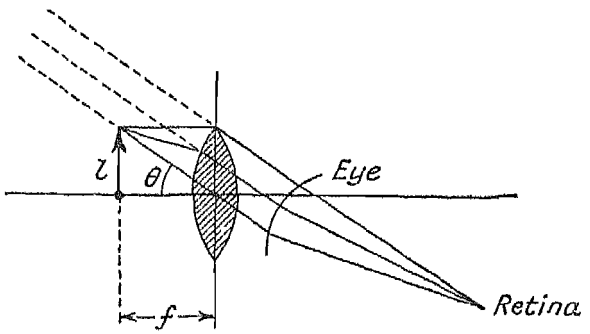


FIG. 3.

lens is expressed in millimeters, the distance of distinct vision is taken as 250 mm.

The magnification formula can be obtained in a rather more orthodox manner, as follows (Fig. 3):

We know that the magnification is equal to:

$$\frac{\text{Angle subtended by the virtual image seen through the lens}}{\text{Angle subtended by the object seen by naked eye at distance of 10 inches.}}$$

(10 inches being the distance of distinct vision, D_v.)

If a small object is placed in the focal plane of a short focus lens held in front of the eye, the virtual image so formed is seen apparently at an infinite distance. The inclination θ of the rays (Fig. 3) depends on l and f, and the angle subtended by the image is now represented by the ratio:

$$\frac{l}{f}$$

Substituting in the expression given above and noting that the

angle subtended by the object at the distance of distinct vision, with the unaided eye is $\frac{l}{D_v}$

then:

$$\text{Magnification} = \frac{\frac{l}{f}}{\frac{l}{D_v}} = \frac{D_v}{f}$$

Thus a lens having a focal length of 2 inches will magnify

$$\frac{10}{2} = 5 \text{ diameters.}$$

A lens with a focal length of $\frac{1}{2}$ inch will magnify

$$\frac{10}{\frac{1}{2}} = 20 \text{ diameters.}$$

A 50-mm. lens will magnify $\frac{250}{50} = 5$ diameters.

COMPOUND MICROSCOPE

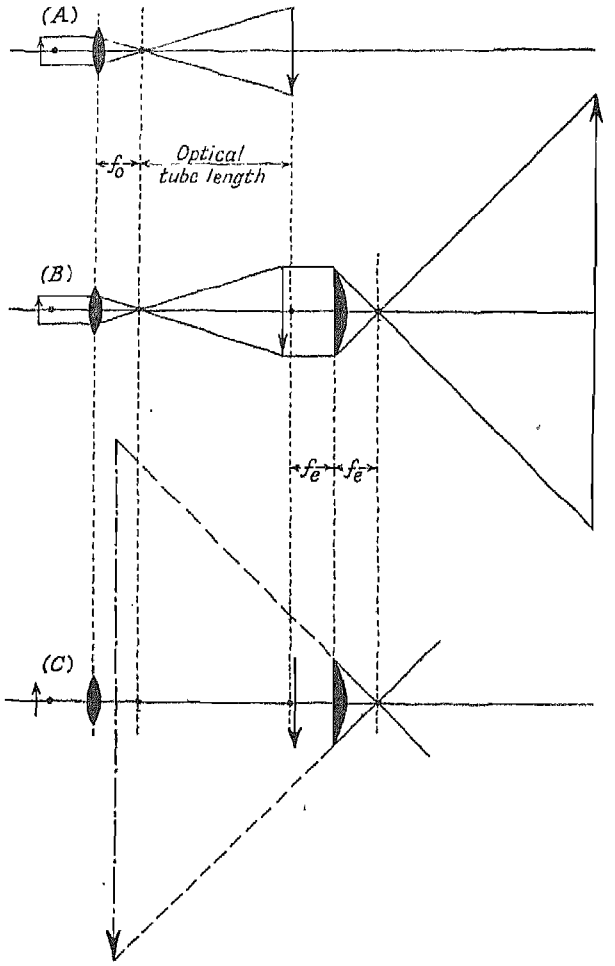
There is obviously a limit to the degree of magnification which can be obtained by means of a single lens.

A lens, however, can be arranged so as to give a *real* image of the object, and this image will be a magnified image provided the object lies between the principal focus of the lens and a point twice that distance from the lens (where image and object are equal in size). The closer the object is brought to the principal focus of the lens, the larger the image will be. Since the image is real, it can be further magnified by means of a second lens. As far as this lens is concerned, the real image formed by the first lens is indistinguishable from a true object, and it will therefore give a new and further magnified image which may be real or virtual.

What happens is shown (in a simplified form) in Fig. 4.

The first lens is the *object glass*, or objective of the instrument, and the second is the *eyelens* or *eyepiece*.

The object glass projects a primary image of an object lying in the plane of its front or lower conjugate focus upon the plane of its upper conjugate focus. This image, which is real and inverted, lies in the plane of the lower conjugate focus of the eyelens. It follows that a secondary image will be formed at its corresponding



(*A*) Object glass alone. Formation of real, inverted, primary image in focal plane of eyelens.

(*B*) Eyelens forming real, erect, secondary image as for projection or photography. Primary image is just outside focal plane of eyelens.

(*C*) Eyelens forming virtual, inverted, secondary image as for visual observation. Primary image is just within focal plane of eyelens.

FIG. 4.—The position of the eyepoint or Ramsden circle is where the eyepiece forms the image of the aperture of the object glass slightly above the outer focal plane of the eyepiece.

upper focus. This secondary image can be thrown on a screen and thus become visible.

If the eye is now placed where it can pick up the rays from the eyepiece, and the distance from object to object glass slightly readjusted to bring the primary image just within the principal focal plane of the eyelens, an enlarged virtual image will be seen, on the same side as the object, and apparently located at a distance of 10 inches (distance of distinct vision) from the eye.

It will be noted that in the case of the *real* image *two* inversions have taken place, so that it is *erect* when seen on a screen. In the case of the *virtual* image, there is only one inversion so that the object is shown *inverted* in the field of view.[1]

MAGNIFICATION Eyepiece.—This depends on the distance D from the screen on which the secondary image is formed, to the eyepiece, and the focal length of the latter.

The *eyepiece* magnification will be $\frac{D}{fe}$ and, in the case of the virtual image, $D=D_v$ (distance of distinct vision) so that the eyepiece magnification becomes $\frac{D_v}{fe}$ or $\frac{10}{fe}$ in. or $\frac{250}{fe}$ mm., just as it was in the case of the simple lens, page 6.

Total.—The total, or overall magnification, is the product of the magnifications of object glass and eyepiece.

[1] Students are sometimes puzzled by the following apparent paradox. If an object is focused visually, the instrument is obviously so adjusted as to give a virtual image. In other words the primary image formed by the object glass must lie within the principal focus of the eyelens. The final image seen by the eye is then virtual and inverted.

If a screen is now placed at a suitable distance from the eyepiece, *the adjustments of the instrument remaining unaltered*, it will be found that a real erect image can be obtained.

Apparently, therefore, the instrument gives a virtual and a real image simultaneously, but this is an impossibility because the primary image cannot be both within and without the principal focus of the eyelens at one and the same time.

This would be perfectly true if the object were infinitely thin, a true optical section or plane. Since the object has depth, however, the primary image is also a space image. Moreover it can be shown that the magnification in depth is equal *to the square* of the linear magnification.

If then we consider two optical sections $0{\cdot}1\mu$ apart, their images will be practically identical and the eye will see no recognisable difference between them. If we assume an initial magnification of 100 diameters, the corresponding sectional planes in the primary image space will be $100\times100\times0{\cdot}1\mu$ apart, or 1 mm. apart, so that there is an ample margin for one of these sections to be within, the other without, the principal focus of the eyelens, and the resulting virtual inverted and real erect images will appear identical to the observer though they are in fact separate images formed by separate optical sections through the specimen.

Objective.—The magnification of the object glass is obtained as shown in Fig. 5.

$$\text{Magnification} = \frac{I}{O} = \frac{I}{AB} = \frac{l}{fo}$$

In other words, the magnification is found by dividing the distance from the focal plane to the image, by the equivalent focal length of the lens system.

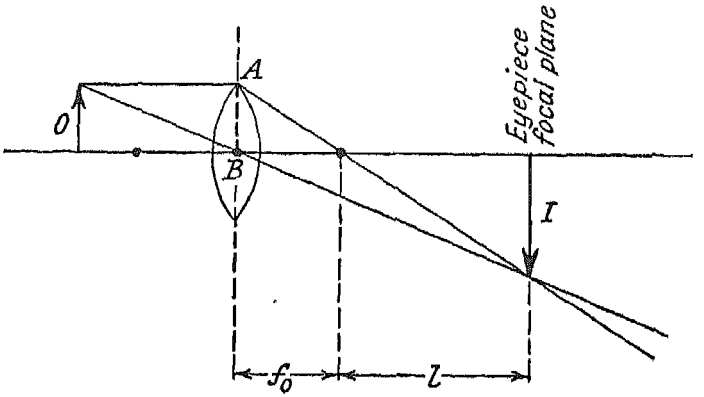


FIG. 5.

Optical Tubelength.—In a microscope the *optical tubelength* is defined as the distance between the upper principal focal plane of the object glass, and the lower principal focal plane of the eyepiece, where the primary image is formed.

Thus, in the above expression l=optical tubelength, and the magnification of the object glass is given by:

$$\text{Magnification (object glass)} = \frac{\text{Optical tubelength}}{fo}$$

Mechanical Tubelength.—There is often confusion between the optical tubelength and the *mechanical tubelength*, which is the distance between the shoulder of the object glass, where it joins the nosepiece, and the upper end of the bodytube (or drawtube where there is one) against which the eyepiece flange rests. It is easy to understand that since the object glass is not a simple lens, but consists of a complex combination of lenses, neither the position of the optical plane nor that of the back focal plane can be determined by the user.

Some constant, easily measured quantity such as the mechanical tubelength becomes necessary as a convenient standard for the

approximate designation and estimation of magnification in practice.

Thus, the *mechanical* is substituted for the optical tubelength when defining the magnifying power of an object glass. They do not, in fact, differ very widely.

There are two standard mechanical tubelengths, the older 10-inch tube, and the modern 160-mm. tube.

Focal Length of Object Glass.—An object glass described as a 2-inch lens will therefore give a primary magnification of:

$$\frac{10}{2} = 5 \text{ diameters on the long tube}$$

or $$\frac{160}{50} = 3 \text{ diameters approximately on the short tube.}$$

The 10 inches here refers to the mechanical tubelength and *not* to the 10 inches distance of distinct vision in the corresponding expressions given for a simple lens, or an eyepiece. The 10-inch tubelength of older instruments and the least distance of distinct vision, being both equal, often confuse the novice. This is no mere coincidence. Ten inches were chosen as the length of the tube in order that the virtual image, seen at the least distance of distinct vision, should appear approximately level with the stage.

Object glasses are always described by makers in terms of their focal length (though some modern objectives are marked with the magnification instead), but it is important to note that the value given is *not the true focal length* of the combination, but an entirely fictitious figure representing *the focal length of a simple lens which would give equivalent magnification.*

Magnification of Eyepiece.—Since it acts as a simple magnifier, enlarging the primary image, the magnification of the eyepiece does not depend on the optical or mechanical tubelengths, but on an independent and constant quantity, the distance of distinct vision.

Consequently the magnification of an eyepiece is exactly determined if we know its focal length. Usually makers prefer to mark the actual magnification on the eyepiece. This is a comparatively recent, and by no means universal practice. The older eyepieces, and some modern ones, are marked with letters (A, B, C, etc.) or figures which mean very little to the user. We will return to this point later.

Field Lens.—The eyepiece does not consist of a single lens, but is composed of a combination of two lenses, the eyelens and the field lens, together forming a single optical unit. The sole object of the field lens is to collect the rays from the object glass so as to form a real image of convenient size and adequate brilliancy in the focal plane of the eyelens.

The combination, however, also corrects certain defects which would be present in the image if a single eyelens were used.

Obviously the simplified arrangement shown in Fig. 4 would be very unsatisfactory. Any defects in the image formed by the object glass would be magnified by the eyepiece, and if a single lens were used as an object glass, these defects would be such as to make the formation of a clearly defined image impossible.

ABERRATIONS

These defects of simple lenses are known as aberrations, and these are of several kinds. The object glass is in fact a combination of lenses so computed as to correct aberrations to a lesser or greater extent, according to the perfection of the combination, the number of its component units, and such considerations as the work for which it is required and the cost of manufacture.

Spherical Aberration.—Spherical aberration is inseparable from the use of lenses with spherical surfaces. The rays passing through the periphery or outer portion of the lens focus on the axis at a shorter distance than those passing through the more central portions of the lens. If, by suitable correction, the rays are approximately brought to a common focus, the lens is said to be *aplanatic*.[1]

Spherical aberration can be avoided by using lenses with aspherical surfaces. Such lenses are much more difficult to grind, however, so that this method is not used in practice.

Instead of using a plain convex lens, we can use a bi-convex lens of shorter focus combined with a negative meniscus so computed as to restore the focus to its original value. Correction is thus obtained because the extra thickness of the meniscus at the periphery, and corresponding thinness on the optical axis enable the computer to obtain accurate compensation. The glass used for the meniscus has a *refractive index* which is different from that used for the bi-convex component. A certain additional spherical

[1] Spherical aberration and aplanatism have nothing whatever to do with the flatness, or otherwise, of the field.

aberration is introduced by the cover glass over the object, and must be allowed for when correcting the lens (see pages 24 and 108).

Chromatic Aberration.—This also means that different rays, passing through the lens, focus at different points, but in this case the difference is due to the different paths followed by light of different wavelengths, or in other words, different colours. The violet will focus nearest the lens, then the blue, the green, the yellow, and farthest of all, the red. This results in colour fringes, because only one colour can be sharply focused at a time in any one plane. An improvement is at once apparent if a convex crown lens and concave flint lens are combined. The four radii and different *dispersive* properties of the two glasses enable the computer:

(1) To bring the central and marginal rays of one chosen or "preferred" colour (usually green) to focus at the same point, thus removing spherical aberration for that colour.

(2) To cause the rays of other colours to concentrate very nearly upon this point, thus achieving partial correction of chromatic aberration.

The lens is now said to be *achromatic.*

If now a similar correction is made for a second colour, the improvement is considerable, and such object glasses are known as *semi-apochromats* or fluorite lenses.

An extension of the corrections to three colours gives an almost perfectly corrected lens, known as an *apochromat.* Generally speaking, achromatic lenses, or at most semi-apochromats, are entirely satisfactory for all visual work. For highly critical work, and particularly for photomicrography, apochromatic lenses are essential if the best results are to be obtained. Here, of course, the question of cost is an important consideration. We will return to this point later.

Inequality of Colour Magnification.—A second form of defect is due to the fact that different colours undergo different degrees of magnification, the blue being magnified more than the red. The effect of this is to superimpose a number of images of different colours and slightly different sizes, thus impairing definition.

In the case of low and average power achromatic lenses, this defect is corrected within the combination, but in the case of apochromatic lenses, it is corrected by means of a special or *compensating eyepiece* so computed as to magnify red more than blue.

Coma.—This is a defect confined entirely to rays coming from points lying outside the axis of the lens. It is not easy to define

and it will be sufficient to mention that it is due to non-fulfilment of the sine-law. Coma has always been eliminated in a properly computed lens.

The following table gives a general summary of the various forms of aberration and the corresponding corrections (Fig. 6).

Illuminators.—Light is supplied to the instrument from beneath the stage (in the case of transmitted illumination) by means of an illuminator which is itself an optical combination often consisting of several lenses. The functions of the illuminator, and the different types used, will be described in a later chapter. They are referred to here only in connection with their corrections.

For most purposes, an *aplanatic* condenser is all that is required. The illuminator does not need to be as highly corrected as the object glass, for obvious reasons.

In order to take the fullest possible advantage of such highly corrected combinations as the apochromats, however, it is necessary to use an *achromatic* illuminator, or even one so well corrected that it approaches the perfection of the object glass itself. Watson's Holoscopic Condenser is of this type, and there are others.

It is preferable to use an achromatic illuminator, generally speaking, with the higher power achromatic lenses.

MAGNIFICATION

Range and Limitations.—Returning now to the more practical aspects of magnification, we find that the total or overall magnification is the product of the magnifications of the object glass and eyepiece.

Any given overall magnification can thus be obtained in several ways.

Any object glass with a high magnification may be combined with an eyepiece of low magnification, and vice versa.

It will be seen from the subject-matter of the next chapter that resolution, or the power of the instrument to reveal fine detail, does not depend on magnification so much as on certain other characteristics such as numerical aperture.

Empty Magnification.—It follows that unless the fine detail is there to start with—has already been resolved by the object glass—no amount of subsequent magnification will reveal it. There is no point whatsoever in magnifying the primary image beyond the stage when the finest detail resolved is comfortably

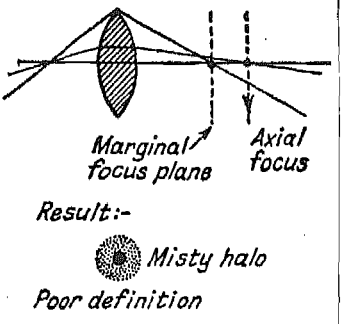
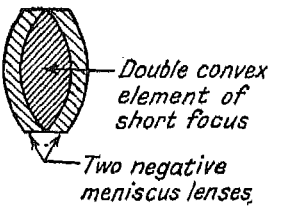
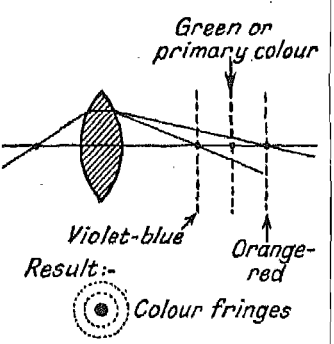
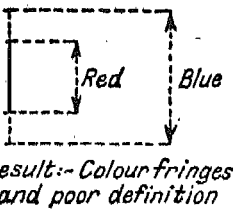
Type.	Effect.	Correction.
Spherical. Marginal rays refracted more than rays nearer optical axis.		*Aplanatic* lenses. (1) Use of aspherical lenses. Costly and difficult. (2) Use of compound lenses of different refractive index.  Compensation is due to greater thickness of negative lenses in marginal zones.
Chromatic. (1) Different colours are *unequally refracted.*		(1) Use of monochromatic light. (2) *Achromatic* lenses. Combining glasses of varying refractive index and *degrees of dispersion.* Correction: Blue-violet and orange-red combined in pairs. Pairs brought closer to focal point of green (primary colour). (3) Coincidence of all three foci, *Apochromatic* lenses. Semi-apochromats are intermediate.
(2) Different colours are *unequally magnified.* Blue more than red.		(1) Corrected within objective for moderate power achromats. (2) Defect *neutralised by* means of *compensating eyepiece* for apochromats. Eyepiece magnifies red more than blue.
Coma. Non-fulfilment of sine-law.	Variable. Side-flare and poor definition.	Should not occur in properly computed lenses.

FIG. 6

ABERRATIONS

seen by the eye, clearly, and without strain. To enlarge the image beyond this is "empty magnification".

Since the magnification of an object glass, its focal length, and numerical aperture are interrelated, the correct procedure is to select an object glass the characteristics of which are sufficient to resolve the detail required, and then to choose an eyepiece that will enlarge the primary image sufficiently to render such detail clearly visible. This will be discussed later.

Usual Standards.—Object glasses are manufactured with focal lengths extending from some 4 inches, giving a magnification of 2·5 times on a 10-inch tube or 1·6 times on a short tube (more usually the limit here is 32 mm., magnifying 5 ×), down to $\frac{1}{12}$ inch giving a magnification of 120 times on a 10-inch tube. The latter corresponds to a 2-mm. object glass giving a magnification of 80 times on 160 mm. (The 2-mm. lens usually magnifies 90 diameters and is thus nearer 1·8 mm.)

Lenses have been made with focal lengths of $\frac{1}{16}$, $\frac{1}{20}$, and even $\frac{1}{50}$ of an inch, but for reasons which are given at some length in the next chapter they reveal nothing more than a $\frac{1}{12}$ inch of large aperture, and have become obsolete.

Eyepieces range from 5 × to a practical maximum of about 25 ×, though 20 × is more usual.

Maximum Magnification.—It will be seen from this that, given the most perfectly corrected lens of high aperture, the maximum possible magnification will be 2,500 × with the long tube, and about 2,000 × on the short tube. It is doubtful, in actual practice, whether a maximum of 1,500 × can usefully or effectively be exceeded. *Magnifications, of course, are linear and refer to diameters.*

Notes on Magnification and its Determination in Practice.—When attempting to work out the magnification of a given set of object glasses and eyepieces, it is a matter of considerable difficulty to obtain correct results.

The values for object glass and eyepiece magnifications given on these components (they are not even always given) cannot be taken at their face value for the following reasons:

(1) Many manufacturers work to a given range of total (overall) magnifications in round figures so that the separate magnifications of their eyepieces and object glasses vary considerably. All sorts of non-standard magnifications can thus be obtained if the products of different makers are combined, particularly when they are used on a stand for which they are not specially computed.

(2) The focal length given for an object glass may thus merely represent its approximate power when used with a particular set of eyepieces on a particular stand.

(3) It is well to remember here that the focal length so indicated bears no relation to the actual focal length of the combination, but conventionally refers to the focal length of a single lens of equivalent magnification.

(4) The expression:

$$\text{Magnification} = \frac{10 \text{ inches}}{\text{focal length}}$$

is true of an eyepiece, because the eyepiece, being a simple magnifier to which the eye is applied, has a constant magnification depending on the focal length *and the* 10 *inch distance of distinct vision*. The magnification of the object glass varies with the *optical tubelength*, and here, when using the above formula, 10 inches (or 160 mm.) represents the *mechanical tubelength* used as a convenient standard *approximation* for the optical length of the tube, which varies according to the object glass (and eyepiece) in use, particularly if they come from different makers and are computed for use on other stands.

The mechanical tubelength can be easily measured with a ruler, and can be adjusted to the exact value required by means of the drawtube, but it gives no clue to the optical length, defined as the distance between the two adjacent (or inner) focal planes of objective and eyepiece.

This often leads to confusion because, when dealing with a 10-inch tube (the general rule in the older text-books) 10 inches is conventionally taken to represent *two entirely independent quantities*, the least distance of distinct vision, and the approximate optical length of the tube. Unless this is clearly understood, these two quantities, both equal to 10 inches, appear to turn up indiscriminately in formulae where they have no business to be.

The correct expressions are:

For an eyepiece

$$M_e = \frac{\text{Least distance of distinct vision}}{\text{focal length}}$$

$$= \frac{10 \text{ in.}}{f_e} \quad \text{or} \quad \frac{250 \text{ mm.}}{f_e}$$

The length of the tube is immaterial.

For an object glass

$$M_o = \frac{\text{Optical tubelength}}{\text{focal length}}$$

$$= \text{conventionally } \frac{\text{Mechanical tubelength}}{\text{indicated focal length}}$$

$$= \frac{10 \text{ in.}}{fo} \textit{ for the long tube } (fo \text{ in inches})$$

$$= \frac{160 \text{ mm.}}{fo} \textit{ for the short tube } (fo \text{ in mm.}).$$

British and Continental Practice.—To make the confusion worse, continental makers often use expressions which are the *reverse* of these. Thus they use:

$$\frac{\text{Least distance of distinct vision}}{\text{focal length of object glass}}$$

for object glasses, so that their effective magnification on the short tube is the same as for a 10-inch tube, British notation, and:

$$\frac{\text{Optical} = \text{mechanical tubelength}}{\text{focal length of eyepiece}}$$

for their eyepieces.

Obviously the products of the values, i.e. the overall magnifications, are the same for both systems.

Measuring the Magnification.—It follows from these considerations that the exact magnifications of object glasses and eyepieces cannot be readily determined, nor will the values indicated on the components give correct results. It may be mentioned here that the indications on object glasses are sometimes deliberately mis-stated so that the lens may appear to have a larger aperture, for its indicated focal length, than it actually has. Thus a $\frac{1}{2}$-inch may be found to be nearer a $\frac{1}{4}$-inch.

However, an *exact* measurement of the overall magnification is only necessary when:

(1) Making a drawing with the camera lucida or a projection device.

(2) Taking a photograph.

In both these cases, fortunately, the magnification can be easily and accurately measured by substituting a stage micrometer for the object slide and measuring the overall magnification direct, with a ruler, on the paper, or ground glass focusing screen.

When *measuring* objects with a micrometer eyepiece a *comparative* method is generally used, so that in this case also there is no necessity to know the exact magnification in use.

In all other cases, all we require is to have some idea of the order of magnification we are using, and this can be easily obtained by drawing up a table giving the overall magnifications of all possible combinations with the components at our disposal. Such a table can be based on the results obtained by any of the following methods:

(A) *Methods for measuring overall magnifications*

(1) Using a stage micrometer (usually a millimeter divided into tenths, one of the tenths being further divided into hundredths), project its image on a screen. Now measure the image. If the image of a $\frac{1}{10}$-mm. division measures 3 cm., or 30 mm., then the overall magnification *on the screen* is

$$M_s = \frac{30}{\frac{1}{10}} = 300\times.$$

This must now be related to the distance of distinct vision in order to obtain the apparent magnification of the *virtual* image seen at that distance when using the instrument for visual observation.

If the distance from the screen to the outer face of the eyepiece is d, then the corrected total magnification will be:

$$M_T \text{ (of virtual image)} = \frac{10 \times M_s}{d}$$

or, in the above example,

$$\frac{10\times 300}{15} = 200\times$$

assuming the screen to have been 15 inches from the eyepiece.

Here 10 is the *least distance of distinct vision*.

Repeating this process for all available combinations, a table of overall magnifications corrected for the least distance of distinct vision, i.e. corresponding to the use of the instrument for visual observation, is readily obtained.

(2) A quicker method, when the number of combinations is considerable, is as follows:

Using a stage micrometer as before, place a micrometer eyepiece in the tube. This has an arbitrary scale engraved on a small glass disc lying in the focal plane of the eyelens.

Using a 1-inch object glass as a convenient standard, focus the

micrometer on the stage and note the number of divisions on the eyepiece scale lying within $\frac{1}{10}$ mm. on the stage micrometer. Let this number be n_1.

Now measure the overall magnification of the combination 1-inch object glass—micrometer eyepiece by means of method (1).

Let this magnification be M_1 (corrected for least distance of distinct vision).

Now work out the value of the factor $\frac{M_1}{n_1}$.

Using *the same eyepiece* in combination with each object glass in turn, we shall obtain various values for the number of eyepiece micrometer divisions contained within one division of the stage micrometer, n_2, n_3, n_4, etc. The corresponding overall magnifications of these object glasses with that particular eyepiece will now be:

$$M_2 = \frac{M_1 n_2}{n_1} \quad \text{or} \quad \frac{M_1}{n_1} n_2$$

$$M_3 = \frac{M_1 n_3}{n_1} \quad \text{or} \quad \frac{M_1}{n_1} n_3, \text{ etc.}$$

Having once worked out the value of the factor $\frac{M_1}{n_1}$ it is easy, by merely reading off the values of n_2, n_3, n_4, etc., for the different object glasses, to obtain the corresponding overall magnifications without further measurement on a screen, and they will be automatically corrected for the least distance of distinct vision.

It is now only necessary to test the various eyepieces with the inch glass by means of method (1), in order to obtain the overall magnification of every possible combination, with sufficient accuracy for the purpose we had in mind.

(B) *Method for measuring true focal length of object glass*

Remove the eyepiece. Project the image of the stage micrometer on to a screen and measure the magnification M_s with a ruler.

If h is the distance from screen *to stage*

$$\text{then } f_o = \frac{h \times M_s}{(M_s + 1)^2}.$$

(C) *Method for measuring the approximate magnification of an eyepiece*

Having the overall magnification measured under (A), M_T, for any given combination, and, the focal length f_o of the object glass

as measured under (B), we first find the magnification of the object glass from the approximations $\frac{10 \text{ inches}}{f_o \text{ inches}}$ for the long tube, or $\frac{160 \text{ mm.}}{f_o \text{ mm.}}$ for the short tube, where 10 inches and 160 mm. represent the conventional tubelength.

Let this magnification be M_o.

Then the eyepiece magnification $M_E = \frac{M_T}{M_o}$.

Taking the average of several measurements made with different object glasses, a fairly close approximation will result.

The overall length of an eyepiece, from outer lens face to outer lens face, gives some indication of its probable magnification.

Length in inches	2 in.	$1\frac{7}{8}$ in.	$1\frac{1}{2}$ in.	$1\frac{1}{4}$ in.	1 in.
Focal length in mm.	63	50	36	25	17
Magnification	4×	5×	7×	10×	15×

This method is unreliable, except as a first approximation when dealing with an unknown and unmarked eyepiece.

Depth of Focus.—There remain a few points to be mentioned in connection with the optical characteristics of the instrument which rather anticipate on the subject-matter of the next chapter since they depend on the numerical aperture of the object glass. The ability of an object glass to reveal fine detail depends on the angular, or, more properly, the numerical aperture of the lens, or, in other words, on the apical angle of the cone of light entering the lens.

It does *not* depend on magnification except inasmuch as the initial magnification of the lens is related to its numerical aperture, being higher, the larger the aperture. Clearly the finer the detail resolved, the more it must be magnified before the eye can see it comfortably.

Apart from resolution, which will be fully dealt with in the next chapter, the numerical aperture affects the *depth of focus* and the *working distance*.

When taking a photograph with an ordinary camera one may either arrange for everything to be in focus from the foreground to the far distance, or for one particular plane to be in sharp focus, and all other objects indistinct. In the latter case the full aperture of the camera lens can be used, but in the former case a small aperture must be used and this is done by "stopping down" the camera lens diaphragm.

In the same way an object glass of low power and correspondingly low numerical aperture will have considerable depth of focus, whereas a high-power lens of very large aperture will have none at all. With such lenses the depth of focus is so small that it is practically an optical section of the object, and the focus is so sharp that portions of the object lying 1 micron above or below this optical plane are not only indistinct, but quite invisible.

Depth of focus is therefore an important characteristic of the lens and must be related to the purpose for which it is being used. This is particularly important when taking photomicrographs on account of the necessity for combining the degree of resolution, or fineness of detail shown, with the depth over which such detail must remain clearly defined.

The depth of focus is inversely proportional to the numerical aperture; the following table shows how it varies for a given magnification. It is also inversely proportional to the magnification for a given value of the numerical aperture.

NA	Depth of focus in air microns	Depth of focus in medium $n=1{\cdot}5$, microns
0·25	7·9	12
0·50	1·9	3
0·75	0·8	1·3
1·00	—	0·7
1·25	—	0·4

(Martin and Johnson, $df = \frac{\lambda/4}{n.sin^2 u/_2}$, $\lambda = 0{\cdot}50(\mu)$.

Working Distance.—This is closely related to the numerical aperture.

The working distance is defined as the distance between the lowest part of the lens mount and the upper surface of a 0·17–0·18 mm. cover glass when the microscope is focused on an object immediately beneath the cover glass.

The working distance again falls off very rapidly as the power, and hence the numerical aperture, of the object glass increases.

Its practical implications are obvious. A lens with a very small working distance requires very thin cover slips, or it may be impossible to focus the object at all. It is also much more liable to accidental damage.

The working distance bears no true relation to the indicated or

equivalent focal length of the lens for reasons which have already been given (Focal Length of Objectives, page 11).

This is clearly shown by the examples listed in the following table, from which it can be seen that in every case the working distance is much smaller than the indicated focal length.

Focal length indicated		Working distance
inches	mm.	mm.
2	50	30
1	25	14–20
$\frac{2}{3}$	16	8–6
$\frac{1}{3}$	8	2–0·90
$\frac{1}{6}$	4	0·5–0·65
$\frac{1}{8}$	3	0·2–0·40
$\frac{1}{12}$	2	0·1–0·25

The working distances vary considerably from one maker to another, and equivalent lenses can be computed so as to give extra long working distances for special purposes.

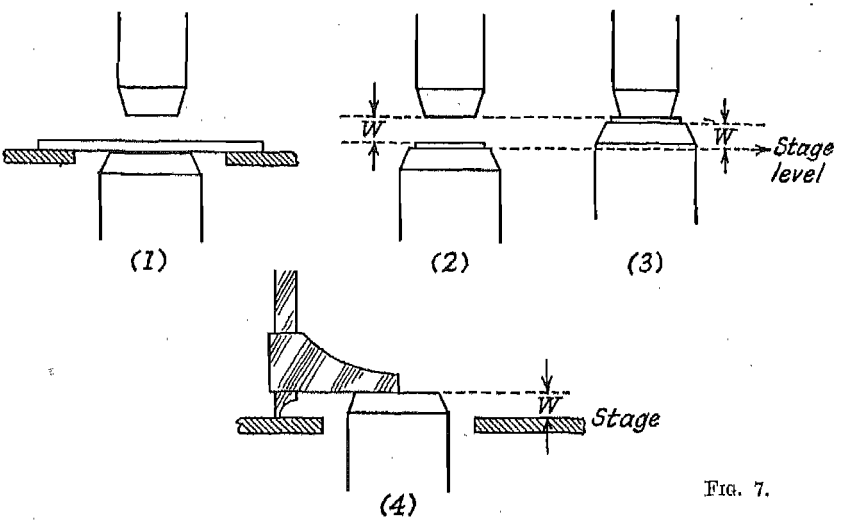


FIG. 7.

Measuring the Working Distance.—It is sometimes convenient to know the working distance of an object glass, and it can be quite easily ascertained as follows (Fig. 7):

(1) Place a glass slide on the stage and rack up the condenser until its front lens just touches the under-side of the slide. Now

remove the slide. The front lens of the substage condenser is now exactly level with the stage.

(2) Make a minute ink mark on one side of a 0·17–0·18 mm. cover slip and place it over the condenser lens, ink mark against the condenser (the instrument being vertical). Now focus the ink mark with the object glass.

(3) Rack up the condenser very carefully until the upper surface of the cover slip just touches the lens mount.

(4) Remove object glass and cover slip. The difference of level between the upper surface of the condenser lens and the stage is now equal to the working distance, and it can be measured by means of a precision depth-gauge.

Effect of Cover Glass Thickness.—The cover glass, being interposed between the object and the object glass, is really part

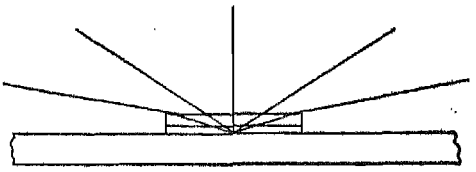


Fig. 8.

of the optical system. When computing an object glass, therefore, allowance must be made for the cover glass and some definite value must be adopted for its thickness. The majority of modern object glasses are corrected for use with a 0·17–0·18 mm. cover glass, but since cover glasses differ considerably in this respect, the microscopist must be familiar with the methods at his disposal which enable him to compensate for such variations.

The effect is unimportant for object glasses of a focal length greater than 16 mm. ($\frac{2}{3}$ inch), but with a 4 mm. ($\frac{1}{6}$ inch) lens, for instance, a variation of 0·01 mm. ($\frac{1}{2500}$ inch) in the thickness of the cover glass will affect the quality of the image appreciably.

In the case of immersion lenses, however, the space between the cover glass and the object glass is filled with a medium having the same refractive index as glass, so that the difficulty does not occur.

The effect of the cover glass is to introduce a certain degree of spherical aberration, owing to the divergence of the rays as they leave the glass (Fig. 8). This additional spherical aberration is taken into account when the computer corrects the lens for

this defect, so that the object glass is perfectly corrected when used with a cover glass of correct thickness, but not otherwise.

There are two methods of compensating for thicker or thinner cover glasses, and in both methods a certain amount of over-correction or under-correction is introduced for this purpose. A third, and more recent method consists in using a "lens corrector".

Correction by means of the Drawtube.—Since the objective is also computed for use with an optical tubelength of definite value,[1] correction can be obtained by altering this length to compensate for the variation in cover-glass thickness. The tube is shortened if the cover glass is too thick, or lengthened if the cover glass is too thin. An adjustment of approximately 10 mm. of the drawtube is required for a 0·01-mm. variation of the cover glass.

A drawtube with a rack and pinion adjustment is ideal for this purpose. If the thickness of the cover glass is known, the drawtube can be set to the correct length as a first approximation by means of the 10-mm. for every 0·01-mm. rule, and final adjustments made by observing the appearance of the image on either side of the focus. This is not an easy matter for the novice, and will be dealt with in detail in a later chapter.

It is important, therefore, when making one's own mounts, to measure the thickness of the cover glass with a micrometer gauge and mark its value on the slide. It saves a great deal of trouble later. To obtain the best results with the highest power dry object glasses the thickness of the mountant must be allowed for when estimating the thickness of the cover glass, so that further adjustment by observing the image will always be necessary even when the thickness of the cover glass is accurately known, unless the preparation is very thin and mounted on the cover glass itself.

Correction Adjustment Collar.—Dry objectives of large aperture are often fitted with an adjustment or correction collar, the effect of which is to bring the back components of the lens nearer to, or remove them farther from, the front components. This again introduces a certain amount of over or- under-correction as a means of compensation.

These collars are usually (not always, unfortunately) graduated in terms of cover glass thickness so that there is again no particular

[1] Any two positions where an object and its image are situated are called a pair of conjugate foci. An object glass can only be absolutely corrected for one particular pair of conjugate foci.

difficulty if the thickness is known. Exact adjustment is obtained by observing changes in the appearance of the image, as stated above.

Lens Corrector.—The "Sir Herbert Jackson Lens Corrector" supplied by Watson screws in between objective and nosepiece. It serves the same purpose as a correction collar and can be used with any object glass. It is a very useful device.

Uncovered Objects.—Since the presence of a cover glass of specified thickness is assumed by the computor when correcting the lens for spherical aberration, it follows that uncovered preparations will give a satisfactory image only with lenses of low power, or oil-immersion lenses, unless further corrected by means of one of the methods mentioned above.

Note.—Detailed instructions covering these correction methods are given in Chapter VII, page 108.

CHAPTER III

NUMERICAL APERTURE AND RESOLUTION

THE ability of an object glass to separate fine detail does not depend on magnification, but on its resolving power. This in turn depends on two factors:

(1) The wavelength of the light used to illuminate the object.

(2) The numerical aperture of the lens.

The second factor in turn depends (Fig. 9):

(*a*) On the angular aperture of the lens, or apical angle of the cone of light entering the lens, defined by the sine of the half-angle *u*, or *sin.u*.

(*b*) On the refractive index *n* of the medium from which light enters the lens.

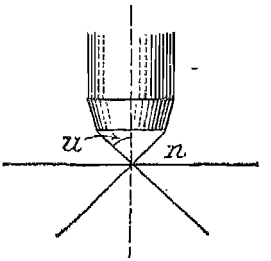


FIG. 9.

The resolving power is thus a function of the wavelength λ, the angular aperture of the lens represented by *sin.u*, and the refractive index of the medium, *n*, from which light enters the lens.

Each of these points will be explained in turn.

Empty Magnification.—The primary purpose of the microscope is to reveal fine detail otherwise invisible to the eye.

The ability to reveal detail or resolution is governed by certain factors outlined above, and is therefore limited.

Theoretically, there is no limit to the degree of magnification, other than aberrations, but there is a very definite limit to resolution.

If we consider two points in the object plane and gradually decrease the distance between them, the distance between the corresponding images of these points will decrease accordingly.

Let us suppose that we simultaneously increase the overall magnification so that the images of the two points remain separate, and are easily perceived as separate points by the eye.

Eventually a limit will be reached beyond which the object glass will be unable to separate the points, and their images will gradually become ill-defined until they finally merge into a single dot. Extra magnification may increase the size of this dot, but can never resolve it into two points.

The object glass has reached the limit of its resolving power.

If we substitute an object glass of the same power, or magnification, but with a higher numerical aperture, we will find that the points have reappeared and are clearly separate. We shall have to bring the corresponding points in the object plane closer together before the image points again become ill-defined, and finally merge.

Increasing the numerical aperture thus decreases the minimum interval between two points or lines on the object which can be separated, or resolved, whereas an increase in magnification cannot in any way effect this.

Resolution is a characteristic quality of the object glass. If the detail has not first been resolved, it does not exist as far as the optical components of the instrument are concerned. The detail will not exist in the primary image formed by the object glass, and cannot therefore be revealed by any amount of magnification beyond that point.

Useful Magnification.—There are two factors to be considered here:

(1) The smallest distance between two points which will enable them to be resolved or reproduced as two separate points by a given object glass.

(2) The two points being correctly reproduced, the minimum magnification required to make them clearly and separately visible to the eye.

Taking the second factor first, it is generally recognised that the human eye can just resolve two points (at a distance of 10 inches) when the distance between them is $\frac{1}{250}$ of an inch, or 100 microns.

It follows that magnification will be *just* sufficient if the smallest interval that can be resolved by the object glass is enlarged until it is equal to 100 microns. Such magnification will be effected partly by the object glass and partly by the eyepiece.

Turning now to the first point, it can be shown that the smallest interval which an object glass of the highest quality can resolve is of the order of 0·20 microns, or 130,000 lines to the inch (approx.)

In order to bring this interval up to 100 microns, when it will become visible to the eye, a total magnification of $\frac{100}{0{\cdot}2} = 500$ is required, but is, theoretically, entirely sufficient.

This may surprise the reader, if he is a novice, because he will be thinking in terms of thousands instead of hundreds. It is a very common error which popular handbooks have perpetuated.

All the detail which the best obtainable lens can resolve will be revealed with an overall magnification of 500 times, and no amount of magnification beyond this can add anything further to the image.

In practice, however, it is well worth increasing this to 1,000 or even 1,500 times, in order that the detail may be more comfortably seen and studied, just as one may make an enlargement from a photographic negative, but no further detail will be revealed.

Since the magnification of the highest power object glass in practical use to-day is of the order of 90 times on a short tube (160 mm.) or 120 times on a long tube (10 inches), it follows that an eyepiece magnifying fifteen to twenty times will cover every requirement. An eyepiece magnifying six times, or at most ten times, is more usual and amply sufficient. Only the best object glasses will stand high eyepiecing without some deterioration in the quality of the image.

Numerical Aperture.—The preceding argument is based on the premises that:

(1) There is a limit of resolution, that is, a minimum interval between two lines or points beyond which an object glass cannot form separate images of them; and

(2) this minimum interval is of the order of 0·20 microns.

The limiting factor is clearly not a matter of magnification. In order to understand this fully we must consider the exact meaning of the term Numerical Aperture on which the resolving power of the combination finally depends.

Antipoint or Diffraction Disc.—The notion of resolution is based on the antipoint theory, which is really a modern version of the diffraction theory of microscopic vision, and its diffraction spectra, referred to under a rather different form in Chapter VI, when dealing with oblique illumination.

According to this theory a true point in the object plane cannot be reproduced as a true point in the image plane.

Owing to the wave nature of light, it is reproduced as a bright dot surrounded by diffraction rings forming a small disc of definite magnitude (Fig. 10). If the interval between two points in the object plane becomes too small, the two corresponding antipoints

will merge, and the points will not be resolved as separate images, whatever the magnification.

Effect of Wavelength.—This will occur when the points are less than half a wavelength apart, hence it follows that the shorter the wavelength, the smaller the interval resolved.

The resolution R, or smallest interval which can be resolved, is thus limited by the factor 0·5 λ, where λ is the wavelength of the light used. This value is sometimes given as 0·61 λ because

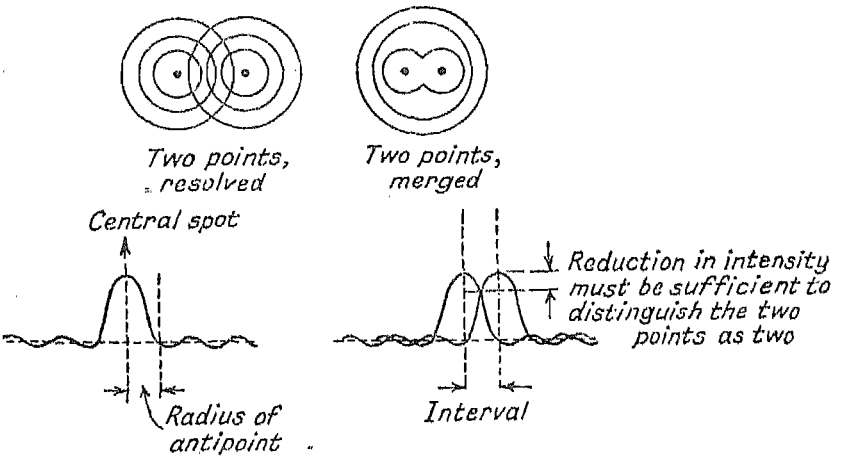


FIG. 10.—Intensity Curves.

the latter value has been found a nearer approximation, but 0·5 λ is more generally used and is then simply interpreted as $\frac{\lambda}{2}$, or half a wavelength. This limit can be raised, and the resolution improved, by means of the second factor mentioned at the beginning of this chapter, namely, the numerical aperture.

Effect of Aperture.—The angle of the light *entering* the lens (and not that which actually produces the image) further determines the ability to resolve because the larger the angle of the cone of light entering the lens, the smaller the radius of the diffraction disc.

The ability to resolve depends on the size of this disc, and hence, for a given wavelength, on the aperture of the lens.

If n is the refractive index of the medium from which light enters the lens, and u the half-angle of the cone of light, then the expression $n \sin. u = NA$ is known as the *Numerical Aperture*.

It has been determined that the radius of the diffraction disc is:

$$r = \frac{0{\cdot}61\lambda}{n\ sin.\ u} = \frac{0{\cdot}61\lambda}{NA}.$$

The resolution is limited by, and therefore equal to, this quantity. If the smallest interval that can be resolved is R, then:

$$R = \frac{0{\cdot}61\lambda}{NA} \text{ for any given lens.}$$

If the value $0{\cdot}5\ \lambda$ is used, the expression becomes $R = \frac{\lambda}{2NA}$.

The resolution will improve, therefore, and the interval resolved R will be smaller:

(*a*) If the numerator is small, or the wavelength short.
(*b*) If the denominator is large, or $n\ sin.u$ is large.
(*a*) Since the wavelength λ is restricted in practice to certain definite values, the resolution will depend primarily on:
(*b*) The term $nsin.u$, or numerical aperture.

This term can be increased:

(1) by increasing n;
(2) by increasing $sin.\ u$, or u.

(1) An increase of n, the refractive index of the medium from which the light enters the lens, is obtained by the use of *immersion lenses* with which the space between the face of the front lens and the cover glass over the object is filled with a drop of immersion fluid, usually cedarwood oil, having a refractive index approximately equal to that of glass. The object is itself mounted in a medium such as Canada balsam of similar refractive index. If in addition the illuminator is oiled to the under-surface of the glass slide, the rays of light travel throughout in a homogeneous medium of high (and constant) refractive index. This condition is known as *homogeneous immersion*.

(2) Full resolving power can be achieved only if sin. u is a maximum, or equal to unity. This means that $u = 90°$. In other words, the lens must gather radiation over 180°, or a complete hemisphere. This is, of course, impossible in practice, but the best lenses take in a very wide cone of light. Apart from other considerations, the value of u is limited by spherical aberration.

It will now be shown that conditions (1) and (2) are interrelated, while the limit for the expression $n\ sin.\ u$ is the value of the refractive index used for the optical components of the lens.

Effect of Immersion Fluid on Angular Aperture.—We can increase the angle of aperture 0 of a lens (Fig. 11) to the theoretical maximum of 180°, a complete hemisphere.

This is impossible in practice, but by introducing a medium of higher refractive index between the object glass and the object,

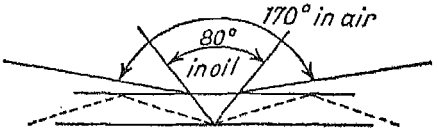


Fig. 11.

we increase n, and enable the lens to collect rays which would otherwise be lost by refraction or total reflection. It follows that *an equivalent pencil of rays can be collected by a lens of lower angular aperture than before.*

It also follows that we may then further increase the angular aperture, thus collecting an even wider pencil of rays.

The equivalent of 180° in air is only 97° in water.

A water immersion lens of 97° angular aperture will therefore collect a pencil of rays equivalent to that collected by a lens of 180° angular aperture working in air.

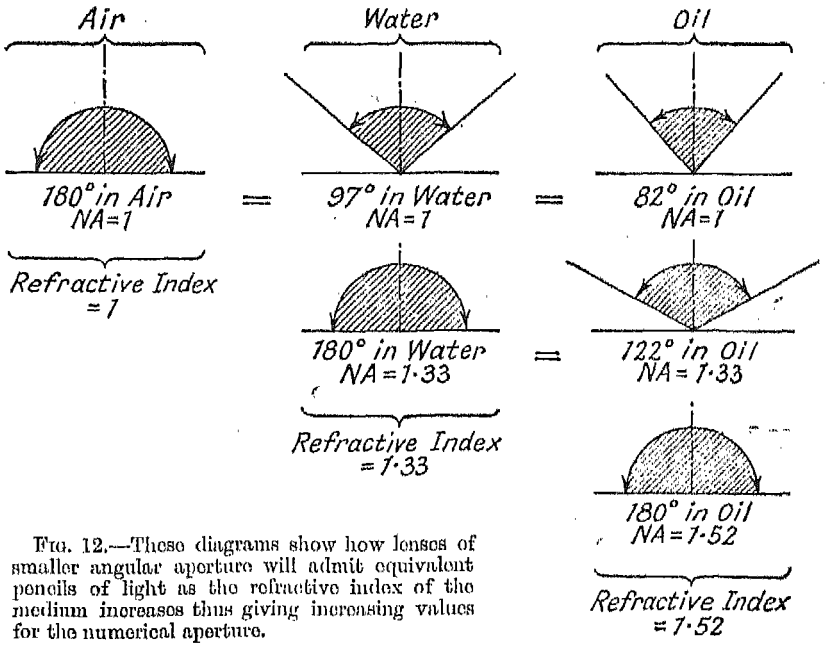


Fig. 12.—These diagrams show how lenses of smaller angular aperture will admit equivalent pencils of light as the refractive index of the medium increases thus giving increasing values for the numerical aperture.

This gives us a fresh starting-point, so to speak, so that we can now increase the angle of aperture in water to a further theoretical maximum of 180°, *thus collecting a pencil of rays actually greater than the theoretical limit or complete hemisphere in air* (Fig. 12).

Having reached this new limit, we may substitute cedarwood oil for water. The pencil of rays collected by 180° in air, or 97° in water, can be collected with only 82° in oil.

The 180° limit in water is reached with an angular aperture of only 122° in oil, and we can thus still further increase resolution by working as close as practically possible to 180° in oil.

This is the final limit, because this medium has the same refractive index as the usual optical glass. Special object glasses have at times been made of extremely dense glass, having a refractive index of the order of 1·65. Using bromide of naphthalene ($n=1{\cdot}64$) as an immersion fluid, the resolution is increased still further.

Aperture and Numerical Aperture.—The term Numerical Aperture is used instead of the angle of aperture in order to obtain an expression equally applicable without further qualification to all immersion media.

For the reasons mentioned above, when giving the angle of aperture of a lens, the nature of the medium must be specified in order that the resolution may be determined, whereas numerical aperture is a criterion independent of the medium, in relation to which the performance of any lens can be assessed.

By including the refractive index of the medium, the equivalent angular apertures of 180° in air, 97° in water, and 82° in oil, all correspond to a numerical aperture equal to unity[1] (Fig. 13).

The angle of aperture is replaced by the sine of the half-angle, and this is multiplied by the refractive index of the medium. As far as air is concerned, n being equal to 1, the numerical aperture is merely the sine of the half-angle of aperture. For mediums of higher refractive index, the introduction of n includes the additional factor required for the true comparison of lenses of different aperture on a common basis, or scale of performance.

[1] Where $NA=n \sin.u$ and $u=\frac{\theta}{2}$ or half-angle of aperture, then:

for Air, $n=1$, $NA=\sin.\frac{180}{2}=1{\cdot}0$

for Water, $n=1{\cdot}33$, $NA=\sin.\frac{97}{2}\times 1{\cdot}33=1{\cdot}0$

for Oil, $n=1{\cdot}52$, $NA=\sin.\frac{82}{2}\times 1{\cdot}52=1{\cdot}0$.

It follows that the maximum, theoretically obtainable, or full 180° of *angular aperture* will correspond to a *numerical aperture* on this scale equal to unity for air, 1·33 for water, and 1·52 for oil.

In other words, the theoretical limit of the numerical aperture is the refractive index of the immersion medium used, so long as this value is at most equal to that of the glass from which the optical components are made.

Order of Magnitude of the Limit of Resolution.—Having dealt with the first point mentioned on page 29, and shown that there exists a limit to resolution, it only remains to deal with the second point, and show that the smallest interval that can be resolved is of the order of 0·20 microns.

Applying the formula $R=\frac{0{\cdot}61\lambda}{NA}$ and assuming a wavelength, $\lambda=0{\cdot}49$ microns for blue light, and an object glass of high numerical aperture, $NA=1{\cdot}40$,

then $R=\frac{0{\cdot}61\times0{\cdot}49}{1{\cdot}40}=0{\cdot}20$ microns.

The limit can be improved by using light of a slightly shorter wavelength and a lens of slightly higher numerical aperture, but the values given above represent the limits usually found in practice.

Ultra-Violet Light.—The resolution can be further increased by the use of ultra-violet light, but quartz optical components must then be used throughout, and the image cannot be seen, though it can be photographed. A wavelength of 0·20 microns can be used, and Zeiss supplied an object glass known as a "monochromat" which had a maximum immersion aperture of 1·25 *NA*. The smallest interval which can theoretically be resolved with this combination is of the order of 0·1 microns.

FURTHER LIMITS OF RESOLUTION

With *visible* light, the limit is reached with a lens of 1·6 *NA* and $\lambda=0{\cdot}4$ micron, giving a limit of resolution of about 0·12 microns.

The *NA* of ultra-violet light quartz objectives is so far limited to about 1·3 for use with water immersion ($n=1{\cdot}33$), and such an objective gives a resolution of 0·1 micron. The only hope of improvement with ultra-violet light over visible light (in conditions where monobromo-naphthalene or some similar immersion

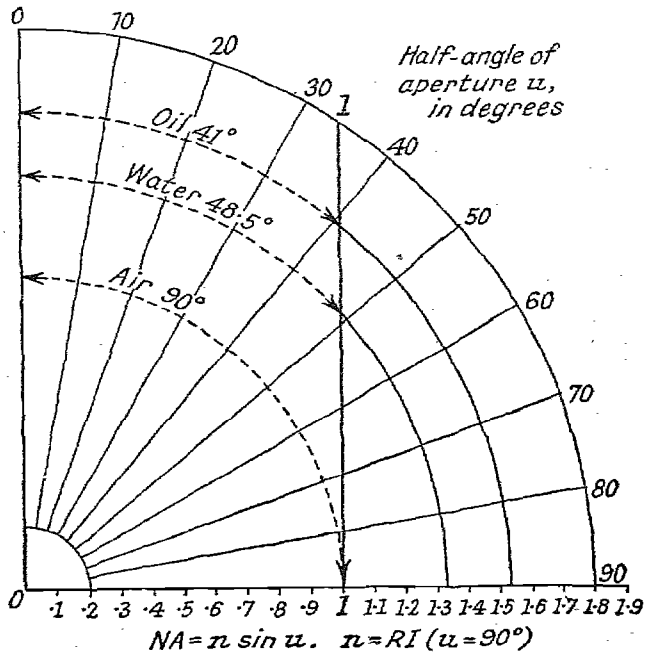
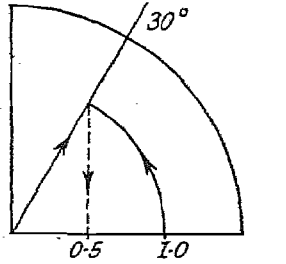


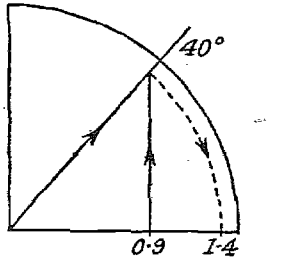
FIG. 13.—Angle of aperture Numerical aperture, and Refractive index.



(1) Given the half angle of aperture, u, and the refractive index of the medium, find NA.

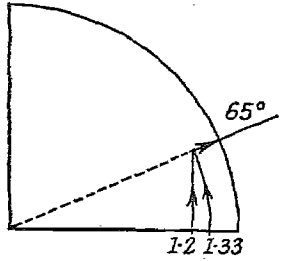
Example: u=30°, RI=1.

Take intersection of unity arc with 30° radius, drop perpendicular, NA=0·5.



(2) Given half-angle of aperture and the NA required, find RI.

Example: u=40°, NA required is 0·9. Take intersection of 40° line with vertical through 0·9, draw arc from this point to base line, RI=1·4.



(3) Given the NA required and RI of medium, find half angle of aperture.

Example: NA=1·2, medium is water, RI=1·33. Take intersection of arc through 1·33 with vertical through 1·2, angle is 65°.

fluid could be used) would be in the production of a quartz objective of higher NA.

The electron microscope, with an accelerating voltage of 50,000 V., and a corresponding wavelength of $5{\cdot}5 \times 10^{-6}$ micron, gives a limit of resolution of $1{\cdot}4 \times 10^{-4}$ micron, or over 1,000 times less than the limit for light. The Universal type has a resolution guaranteed better than 100 Ångstrom units (0·01 micron). This means that particles of one-millionth of an inch can be easily seen. The depth of focus is from 10 to 25 microns, and photographic enlargements can be made up to 100,000 diameters (overall).[1]

Conclusions.—It should now be evident that the true criterion for the performance of an object glass is the value of the numerical aperture, and not the "power" or magnification.

An object glass of high power and short focal length may not have the same resolving power as a lens of lower power. It all depends on the numerical aperture. Naturally the magnifying power of a lens bears a definite relation to the numerical aperture. The magnification obtained within the object glass must be sufficient to ensure that the detail can be seen comfortably by the eye without the necessity for undue magnification by the eyepiece.

For this reason there is no point in unduly increasing the numerical aperture of a low-power lens. There is a further limitation here because, with low-power lenses, high numerical apertures require back lenses of very large diameter. The diameter of the back lens is limited by the diameter of the standard microscope tube.

Equivalent focal length			NA Achromatic	NA Apochromatic
On 160 mm. tube	mm.			
Initial Magnification 5x	32	(1⅓″)	0·10	—
7x	24	(1″)	0·15	—
10x	16	(⅔″)	0·28	0·30
20x	8	(⅓″)	0·50	0·65
40x	4	(⅙″)	0·65	—
—	—	—	0·85	0·95
60x	3	(⅛″)	—	0·95–1·30
90x	2	(1/12″)	1·25	1·40

[1] G. Parr: The Electron Microscope; Association for Scientific Photography, Proceedings, November 1944.

The numerical aperture is also limited by the degree of correction achieved within the objective. Apochromatic lenses therefore generally have a higher numerical aperture than achromatic lenses of equal magnification.

The values for numerical apertures are now more or less standardised, and do not vary appreciably from one maker to another. They are generally as shown in the table on page 36.

The following table gives the value of the resolution in function of the numerical aperture for apple-green light:

$$\lambda = 0{\cdot}550 \text{ using the formula } R = \frac{\lambda}{2NA}.$$

Resolution in terms of Numerical Aperture

$$\lambda = 0{\cdot}550 \qquad R = \frac{\lambda}{2NA}.$$

Numerical Aperture	Interval resolved μ	Lines per inch, approx.
0·1	2·75	9,200
0·2	1·37	19,000
0·3	0·92	28,000
0·4	0·69	37,000
0·5	0·55	46,000
0·6	0·46	55,000
0·7	0·39	65,000
0·8	0·34	75,000
0·9	0·31	82,000
1·0	0·275	92,000
1·1	0·25	102,000
1·2	0·23	110,000
1·3	0·21	120,000
1·4	0·20	127,000
1·5	0·18	140,000

Permissible Magnification.—From the considerations mentioned earlier in this chapter it can now be stated that the maximum magnification which is useful, effective, and compatible with a perfectly defined image is of the order of 1,000 times the value of the numerical aperture (see chart, pages 106 and 107).

Other Factors Affected.——The following characteristic qualities of an object glass depend on its numerical aperture:

(1) RESOLUTION.—This is proportional to the value of the numerical aperture.

(2) Brilliancy of the image. This is proportional to the *square* of the numerical aperture.

The intensity of the illumination is also *inversely* proportional to the square of the magnification (since the *area* illuminated depends on the square of the field diameter). It follows that the brilliancy of the image will remain unchanged if we double the magnification and also double the numerical aperture.

(3) The Depth of Focus.—This is inversely proportional to the numerical aperture, and has been dealt with in the previous chapter. The penetration, or depth of focus, or the number of "optical sections" of an object that can be seen sharply at the same time is very small. The depth of focus decreases very rapidly as the numerical aperture increases. This can be seen from the following table (Martin and Johnson):

NA	Depth of focus in air mm.	Depth of focus in medium $n = 1{\cdot}5$ mm.
0·25	0·0079	0·0122
0·50	0·0019	0·0030
0·75	0·0008	0·0013
1·00	—	0·0007
1·25	—	0·0004

To summarise these remarks:

Resolution is proportional to NA.

Brilliancy is proportional to NA^2 and inversely proportional to M^2 (magnification).

Depth of Focus is inversely proportional to both NA and M, and falls off very rapidly since both NA and M increase together.

Working Distance is a value which falls off rapidly as the initial magnification and NA increase. For given characteristics, however, the value of the working distance varies considerably from one type of lens to another.

Field of View.—The field of view decreases in diameter as the magnification increases but, in a compound microscope, this is a characteristic of the eyepiece—objective combination considered as a whole, since the field-stop in the eyepiece finally limits the field.

Measurement of Numerical Aperture.—The numerical aperture of an object glass being the only true measure of its performance, it is surprising to find that its value is not always given by the maker.

The value is nearly always given for objectives of high quality, particularly the apochromats, and this practice has been extended in recent years to cover all modern lenses of whatever focal length.

When selecting an object glass for any specific purpose, the numerical aperture is the first consideration, and it is therefore important to know, at least approximately, its value for the various object glasses available.

The only accurate methods of measuring the *NA* are the Abbe apertometer and Abbe test plate methods. The Abbe apertometer is an expensive device and its sole use is for measuring apertures. Having such a limited field of application, it is not likely to be used by the average reader. The test plate is a simpler device, and can be used for other purposes, but both methods require skill and a considerable amount of knowledge and experience. The reader will find details of these methods in any of the more advanced text-books.

There is, however, a useful method for determining the approximate *NA* of object glasses given by R. M. Allen (*The Microscope*, Chapman and Hall, Ltd., London).

It is based on the assumption that a certain number of object glasses of known numerical aperture are available.

Using one of these, and focusing an object on a slide, the substage illuminator is adjusted so that the image of the light source is simultaneously in sharp focus in the field of vision.

Now remove the eyepiece and look down the tube at the back lens of the object glass. Slowly close the substage illuminator diaphragm until its edge just appears within the back lens outer circumference. Measure the aperture of the diaphragm with care.

Repeating this process with all object glasses of known aperture, a graph can be plotted showing the values of *NA* against the sizes of the diaphragm aperture. This will give sufficient points to enable the curve to be drawn in with reasonable accuracy.

When an object glass of unknown *NA* is to be tested, all that is required is to proceed as described above, and measure the corresponding diameter of the diaphragm aperture. The value of the numerical aperture can then be read off from the graph.

The principal source of error is variation in the thickness of the slide, and the same slide should therefore always be used for the purpose. The distance from light source to illuminator diaphragm should also be constant.

CHAPTER IV

THE MICROSCOPE, ITS COMPONENTS AND ACCESSORIES

IT is no exaggeration to say that in almost every field of scientific work and industrial research the microscope has become an indispensable tool. In view of this growing demand, it is natural that makers should have incorporated many new and valuable features into their designs. Improvements, during the last fifty years, have been very marked. The optical components have reached a point so near perfection, within the inevitable limitations imposed by theoretical considerations, that it is very unlikely they can be improved any further. The mechanical design of the instrument has undergone many radical changes, the trend being always towards rigidity, stability, and fineness of adjustment.

Whereas earlier instruments were intended to serve any and every purpose, and were consequently provided with a wealth of accessories, the tendency nowadays is to restrict the use of standard instruments to work of a general nature, and to design additional instruments of unorthodox type specially adapted for such specialised applications as petrology, mineralogy, low-power industrial requirements, etc.

The microscope is essentially an optical instrument, and the mechanical design of the instrument is subordinate to, and governed by, the optical requirements. It is for this reason that the earlier chapters have dealt at some length with the instrument's optical principles and characteristics. The mechanical design, the components and accessories, are a natural sequel, and their own principles and characteristics are best understood when related to the optical theory.

To magnify an object, which is the purpose of the microscope, we have to bring a lens, or object glass, to bear upon it, thus forming a real primary image in the plane of the back conjugate focus. We then observe this primary enlarged image with a second lens, or eyepiece, thus substituting for the real image a further enlarged virtual secondary image which the eye perceives apparently at the minimum distance of distinct vision. We shall also have to provide suitable illumination.

(1) In the first place, therefore, the two lens combinations must be rigidly held in their proper relative positions, and accurately centred on a common optical axis. This is the function of the BODYTUBE.

(2) The relative positions of the lens combinations, eyepiece and object glass, must, however, be adjustable to allow for differences between object glasses, and variations of cover glass thickness. This is the function of the DRAWTUBE.

(3) The combined optical unit thus constituted, or compound microscope, must now be accurately focused upon the specimen, and sufficient range must be provided to accommodate object glasses with focal lengths varying from over 32 mm. to under 2 mm. This is the function of the FOCUSING ADJUSTMENTS (coarse and fine).

(4) The specimen must be rigidly held in a plane perpendicular to the optical axis of the bodytube. This is the function of the STAGE.

(5) It is a great convenience to be able to move the specimen smoothly, regularly, and precisely, and to note and measure such motion. This is the function of the MECHANICAL STAGE (scales and verniers).

(6) The specimen must be suitably illuminated. In the majority of cases the object is transparent and examined by transmitted light. This is the function of the SUBSTAGE ILLUMINATOR.

(7) The illuminating cone must be carefully controlled, centred, and focused upon the specimen. This is the function of the SUBSTAGE FITTING (including diaphragm, centring device, and focusing adjustment).

(8) For convenient use with lamps of different types, and with the instrument body vertical or inclined, light must be conveyed from the source into the illuminator. This is the function of the SUBSTAGE MIRROR.

(9) The bodytube, specimen, illuminator, and mirror must be optically aligned and held rigidly in position without flexure or tremor. This is the function of the STAND, BASE AND LIMB (and general design including weight, stability, and rigidity). These are the essential parts and components of the instrument, and their general design is illustrated diagrammatically in Fig. 14.

In addition to these there are a number of extra components and accessories the more important of which will be described in

this and subsequent chapters. Special instruments and devices adapted for such applications as petrology or mineralogy and metallurgy will be dealt with very briefly later.

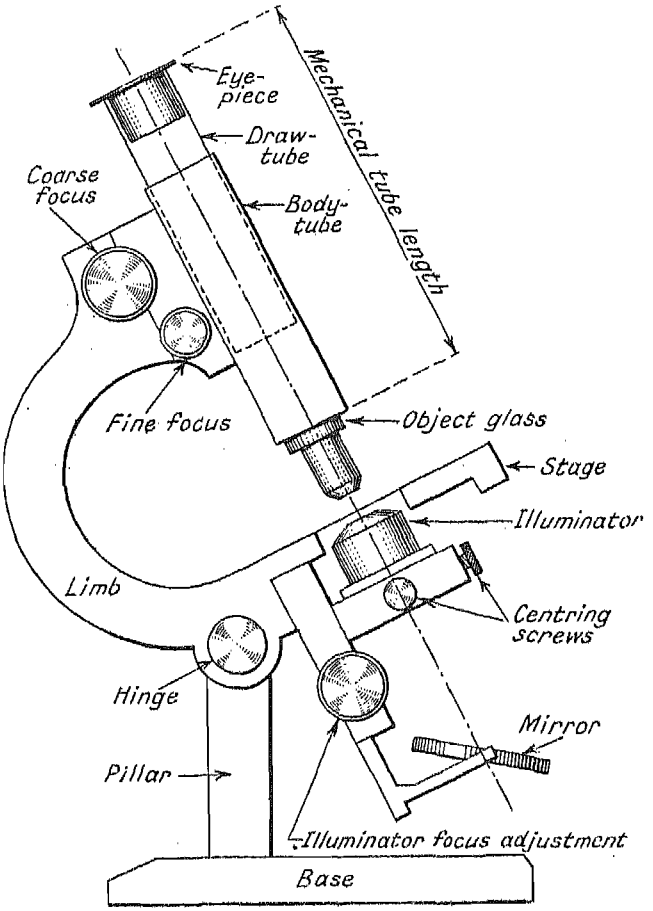


FIG. 14.

Standard instruments and components are so fully described in text-books, and profusely illustrated in makers' catalogues, that only an outline need be given here, with particular emphasis on the purpose and function of these devices in relation to one another and to the instrument as a whole, in accordance with the general principle on which this book is planned.

The main additional components, and their functions, are as follows:

(10) It is necessary to control the beam of rays from the light source. They may need concentration in a convergent beam, or paralleling. The size of the beam will also need control. CONDENSING LENS (and condensing lens diaphragm).

(11) Most work is done with transmitted light, but some specimens are opaque and require illumination from above. VERTICAL ILLUMINATORS.

(12) Other specimens are so transparent or so small that they are almost invisible in transmitted light. They can be brightly illuminated against a dark background. STOP CARRIER. DARK-FIELD ILLUMINATOR.

(13) It is often necessary to measure specimens accurately. MICROMETER EYEPIECE (and stage micrometer).

(14) It is convenient, when changing repeatedly from low powers to high powers to have some means of passing rapidly from one object glass to another. REVOLVING NOSEPIECE. SLIDING CHANGERS.

(15) In addition to the usual lateral and vertical motions of a mechanical stage, it is an advantage to be able to rotate the specimen about the optical axis and to measure the extent of such rotation. ROTATING STAGE (and scale).

(16) Certain objects reveal their structure only when examined in a polarised beam. POLARISER ATTACHMENTS.

(17) For low power work and, under certain conditions, for higher powers, it is of great assistance to use both eyes. Stereoscopic effects result with the lower powers. BINOCULAR INSTRUMENT or EYEPIECE ATTACHMENT.

(18) For microchemical applications, or for determining the nature of minute quantities of a substance, spectroscopic examination is necessary. SPECTROSCOPIC EYEPIECE.

(19) Where a considerable amount of routine examination is necessary, and the instruments are in inexpert hands. COMBINED UNIT ILLUMINATORS.

Such are the main components and accessories. Some are almost essential for serious work, others are refinements, others again apply only to special fields. This aspect is discussed in the next chapter.

(1) **The Bodytube** carries the object glass which is screwed into the nosepiece end. The thread used is now an international

standard, so that all object glasses are interchangeable provided due allowance is made for tube length. The eyepiece slips loosely into the tube at the eye end, in a standard size fitting. There is a telescopic tube, called a *drawtube* within the bodytube, for varying the distance between the object glass and the eyepiece, and a diaphragm to prevent reflections from the inner surfaces of the tubes from entering the eye.

(2) **The Drawtube** is important for three reasons:

(*a*) The bodytube is usually 140 mm. in length, and the drawtube is used to set the length to 160 mm. exactly for lenses corrected for this tubelength. It can in many cases be extended to 250 mm. in order to accommodate lenses corrected for use on the older 10-inch tube. This is sometimes done by means of a second or inner drawtube.[1]

(*b*) The drawtube is used as an adjustment in order to compensate for variations of thickness in the cover glass. The cover glass introduces a certain amount of spherical aberration, and the lens is computed for use with a cover glass of specified thickness. For this purpose the drawtube (the outer one where there are two) is often provided with a rack and pinion adjustment, on the best stands.

(*c*) Within limits, and subject to the above considerations, the drawtube can be used to increase or decrease the overall magnification. The farther it is pulled out, the greater the magnification. *This can only be done within very narrow* limits, except with the lowest powers, or the image will seriously deteriorate, but it is very useful when it is required to adjust the magnification to some simple round figure for measurements, etc.

(3) **The Focusing Adjustments.**—These are of the utmost importance. The coarse adjustment must have a sufficient range to accommodate both low-power lenses of long focal length, and high-power immersion lenses with a working distance of the order of 0·1 mm. It must be sufficiently accurate to enable a medium-power lens to be focused precisely and quickly without using the fine adjustment.

A modern wide aperture 2-mm. oil-immersion lens not only has

[1] The drawtube is also used as compensation for additional fittings introduced between the nosepiece and the objective, such as vertical illuminators, revolving nosepieces, objective changers, Davis diaphragms, etc., the effect of which would otherwise be to lengthen the tube.

a working distance of 0·1 mm., but a depth of focus of not more than 0·0004 mm. Consequently the fine adjustment must be of the very finest quality.

It is usually operated by means of a milled micrometer head so graduated that one division on the scale corresponds to ·002 mm. displacement vertically, and one complete turn moves the object glass up or down 0·1 mm. or $\frac{1}{250}$ inch approximately. Some fine adjustments are provided with a "two speed" device so that they may be rapidly brought to approximate focus.

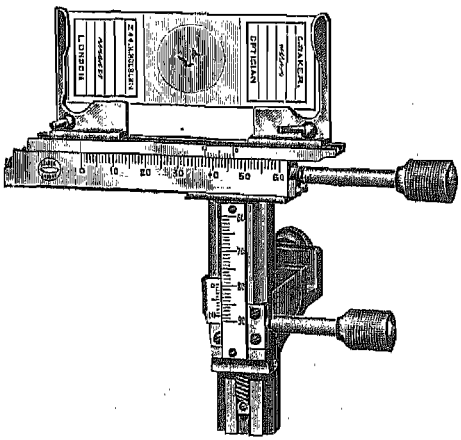


FIG. 15.—Typical detachable mechanical stage (C. Baker).

(4) and (5) **The Stage.**—The first essential is that the stage should be absolutely rigid. It should have no tendency to spring out of focus, when using a high-power lens, under the pressure of the fingers, or when the mechanical stage is being operated.

The next essential is size. A satisfactory stage should measure at least 4 inches each way, and the distance from its centre to the limb should be at least $3\frac{1}{4}$ inches, so that the whole of the contents of a 6-inch petri dish or culture plate can be examined.

If the microscope is placed in a vertical position the slide or slip may be allowed to rest on the stage, but it is difficult to move it evenly unless it is held in some way. Usually, in the simpler forms, a pair of stage clips is provided which fit into two holes in the stage.

A sliding ledge which fits over the bevelled edges of a square

stage is a very convenient device. It is not so convenient as a mechanical stage, but makes a convenient substitute.

A mechanical stage is a device which grips the slide and, by means of two milled heads, moves it smoothly and evenly in either direction. This appliance is almost essential when exacting work is being performed. It enables the whole of the specimen to be systematically examined over its entire surface, and may be provided with scales and verniers so that any part of the specimen can be recorded, and found for further examination at any future time.

There are many types of mechanical stages, some incorporated with the stage itself, and others which can be fitted to existing

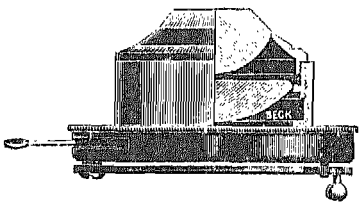


FIG. 16.—Plain Abbe type condenser (Beck).

stages, and may be easily attached or detached. The vertical travel is usually of the order of 1 inch and the horizontal travel 3 inches. The scales read to 0·1 mm. by means of verniers. One advantage of the detachable stage is that it can be entirely removed to leave the stage clear for petri dishes, culture plates, test tubes or large objects.

(6) and (7) **Substage Illuminators and Fittings.**—The substage condenser or illuminator is one of the most important components. We refer to it generally as an illuminator, rather than a condenser, to avoid confusion with the condensing lens or lamp condenser when dealing with illumination.

There are three different kinds of illuminators used for transmitted light.

(*a*) The so-called *Abbe condenser* which consists of two lenses with an iris diaphragm close behind the back lens and a carrier below for stops or filters. It does not focus the rays correctly to one spot nor form a definite image of the source of illumination due to the uncorrected lenses used in its construction. It has a numerical aperture of 1 *NA*. It is sufficient for general purposes.

(*b*) The *achromatic condenser* has the same aperture, but is corrected almost as carefully as an object glass. Freedom from spherical aberration is a valuable characteristic for, while it is possible to converge a solid cone of rays upon a point in the object by means of an uncorrected condenser, it is a necessary condition for this that the source of light be very large, and this condition gives rise to glare. With an aplanatic condenser the source of light may be as small as desired, and these condensers are also achromatic.[1] The condensers are free from spherical aberration to the limit of the aperture, and a cemented triple combination is used to correct the errors of a group of separated single lenses.

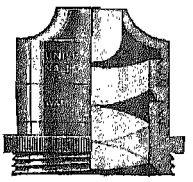


Fig. 17.
Achromatic condenser (Watson's Universal).

(*c*) *Oil-Immersion Condensers or Illuminators.*—Achromatic condensers of the preceding class can also be used as oil-immersion illuminators and are, for this purpose, oiled to the underside of the glass slide. The aperture of the universal condenser of 1·0 *NA* by Watson, for example, will then have an aperture approaching 1·30 *NA*. The numerical aperture of the illuminator must be at least equal to that of the object glass in use, otherwise the effective numerical aperture of the combination being half the sum of the two numerical apertures, will therefore be smaller than that of the objective.

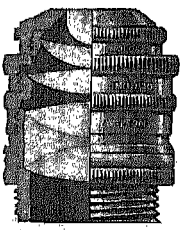


Fig. 18.
Oil-immersion holoscopic condenser (Watson).

Illuminators specially designed for oil-immersion, and for use with high power apochromatic oil-immersion lenses are of even higher quality than the standard achromatics.

The different types of illuminator are made to drop into a tubular sleeve fitting which is provided with some form of focusing adjustment, the most satisfactory being a rack and pinion device similar to that used for the coarse focusing adjustment.

The accuracy of centring of the simple Abbe condenser is not

[1] The *lamp condenser* diaphragm can be closed down until only a very small portion of the field of view is illuminated, but in spite of this, the back lens of the objective will be full of light if the illuminator is truly aplanatic and properly centred and adjusted.

important. The image given is not accurate, and it is generally sufficient to move the mirror slightly till the image of the object remains central when the bodytube is racked up and down.

When using achromatic or immersion illuminators, centring is a matter of great importance, and for this reason the substage illuminator fitting should always be provided with some form of centring device.

(8) **Substage Mirror.**—A double mirror, plane and concave, is fitted to the lower end of the limb, or tailpiece. It swings in gimbals and can be moved in all directions. It has on one side a flat silvered surface, which gives a plane reflection, and on the other a concave surface which concentrates a beam upon a small area of the object when a condenser is not in use, for very low-power work. It is used to direct a beam of light from the light source on to the illuminator. It is important that the plane surface should be parallel-worked, or multiple reflections will result. The plane side of the mirror is always used with illuminators or condensers.

(9) **Base, Pillar, and Limb.**—These have been the subject of much discussion and many modifications. Any design is sound if the instrument is rigid, sufficiently heavy, and stable in any position.

The tripod base is probably the more stable, but it gets very much in the way of the substage fittings when the instrument is vertical or only slightly inclined, and it is liable to slip off the edge of the table with disastrous results.

The horseshoe pattern is preferable from this point of view but relies rather more on its weight and size for stability.

The limb is a solid casting shaped for conveniently lifting the instrument without risk of damage. It is hinged into the top of the pillar so that the instrument can be used at any angle from the vertical to the horizontal.

Such is the microscope, in its modern conventional form, from which there are few radical departures.

ADDITIONAL COMPONENTS AND ACCESSORIES

These are considered in the next chapter from the angle of their relative importance to the user.

All devices (10, 11, 12), connected with illumination, are fully dealt with in Chapter VI.

Rotating stages, polariser attachments, binocular instruments, spectroscopic eyepieces and combined illuminators (15, 16, 17, 18, 19) will all be dealt with as special applications, grouped together in Chapter XI, but only briefly, as an indication of their possibilities.

There remain but two devices in common use, the micrometer eyepiece, and objective changer, to be described in this chapter.

(13) **Micrometer Eyepieces.**—The measurement of objects is important in microscopic work (Chapter VIII) but the same degree of accuracy is not always required. There are therefore different types of micrometers suitable for different degrees of accuracy.

Measurement methods are comparative methods, so that the degree of magnification is immaterial and need not be known.

Briefly, the general principle is as follows:

A glass disc, engraved with an arbitrary scale, rests, scale side down, on the eyepiece diaphragm in the focal plane of the eyelens. The eye will thus see an image of this scale in sharp focus superimposed upon the image of the specimen. The scale is first calibrated against a stage micrometer which consists of a millimeter divided into tenths, one of the tenths being further subdivided into hundredths. The slide with the specimen is then substituted for the micrometer slide, and the size read off on the eyepiece scale.

(*a*) The first and simplest type therefore consists of an ordinary eyepiece with a glass disc and scale resting on the diaphragm.

(*b*) If the observer's vision is abnormal, it may not be possible to keep the scale and specimen in sharp focus simultaneously. Special eyepieces are provided in which the position of the eyelens is adjustable so that the scale can always be sharply focused.

(*c*) For greater accuracy, the filar micrometer eyepiece should be used. The principle is exactly the same, but the reference lines on the eyepiece scale are subdivided by a movable hair-line actuated by means of an external drum graduated into 100 parts so that very small values may be read from the drum.

(14) **Objective Changers.**—There are two types of objective changers, the revolving nosepiece, and the sliding changer. They represent more than just two ways of doing the same thing. Whereas the first is mainly a time-saving device, the second ensures that all object glasses are accurately centred and register exactly as regards the optical axis and the field of view.

The revolving nosepiece is either double, more generally triple, and occasionally quadruple. It screws into the fixed nosepiece of the microscope and each objective in turn can be rotated into the optical axis, thus saving the necessity of unscrewing an objective and screwing another on. This is particularly convenient when a low-power object glass is used for searching and a more powerful glass immediately required for examining fine detail. These nosepieces are made so that no dust can drop back into the object glasses, and they can be safely left attached to the microscope. The extra length of the nosepiece (about 10 mm.) is compensated by setting the drawtube to 150 mm. instead of 160 mm.

The sliding object glass changer consists of an adapter fixed to the nosepiece of the instrument. Each object glass is screwed into a fitting which slips into this adapter. Each fitting is provided with two adjustable abutment screws, usually operated by a watch key, so that once the object glass concerned has been correctly centred, it will always register exactly when slipped into place.

There are many other minor accessories listed in the makers' catalogues, but their value depends so much on the class of work done that the reader will be well advised to gain experience before adding too many of them to his equipment.

Optical Components.—The object glass, or objective, can be of either one of three classes:

(1) *Achromatic.*—In which chromatic aberration has been corrected for two colours, and spherical aberration for one colour.

(2) *Semi-Apochromatic.*—In which fluorite is used for one of the elements, giving a higher degree of correction than in achromats, but not equal to apochromats.

(3) *Apochromatic.*—The finest type of object glass in which chromatic aberration is corrected for three colours, spherical aberration for two colours, and the secondary spectrum eliminated.

Object glasses may further be either of the dry, or of the immersion type. Object glasses of powers exceeding $\frac{1}{6}$ inch or 4 mm. are almost always of the immersion type. The reason for this is that the numerical aperture of a dry lens cannot exceed unity, and is at most equal to 0·95 in practice. The magnification initially required with such an aperture is of the order of 40 diameters, and a 4-mm. apochromat of the finest quality will have this aperture. Unless, therefore, there is some other reason for using a dry lens, there is no advantage in using higher powers dry. The $\frac{1}{6}$-inch (4-mm.) object glass will normally be the highest

power dry lens used, although dry $\frac{1}{8}$-inch (3-mm.) lenses are sometimes supplied.

Immersion lenses, with numerical apertures of 1·2, 1·25, 1·3, or 1·4 *NA*, are computed for use with thickened cedarwood oil having a refractive index of 1·515 approximating the index of the glass used for cover and slide.

Some immersion lenses are corrected for use with glycerine, and some with water. The latter are very useful for the examination of minute living organisms which cannot, of course, be immersed in any other fluid.

A typical set of objectives for general use on a 160 mm. tube will be:

24 mm. 6x
16 mm. 10x
8 mm. 20x
4 mm. 40x
} Dry.

2 mm. 90x Oil immersion.

Eyepieces { 6x, 10x, 20x

This would give a sufficient range for all purposes. The resolution, as we have seen, depends on the numerical aperture, and this again will depend on the quality and class of the objectives.

Characteristics of Standard Objectives (Beck):

			NA	Magnification	Working distance mm.
	Achromatic				
Dry	$2\frac{1}{2}''$	60 mm.	0·07	1·25x	—
	$2''$	50 ,,	0·08	2x	74
	$1\frac{1}{2}''$	32 ,,	0·15 or 0·12	4x	52 or 22
	$\frac{2}{3}''$	16 ,,	0·28 or 0·17	10x	6·5
	$\frac{1}{3}''$	8 ,,	0·5	20x	2
	$\frac{1}{6}''$	4 ,,	0·85 or 0·65	45x	0·6
Oil	$\frac{1}{8}''$	3 ,,	0·95	60x	0·4
	$\frac{1}{12}''$	2 ,,	1·3 or 1·0	90x	0·2
	Apochromatic				
Dry	$1\frac{1}{2}''$	40 mm.	0·16	3x	—
	$\frac{2}{3}''$	16 ,,	0·35	10x	2·5
	$\frac{1}{3}''$	8 ,,	0·65	20x	1·0
	$\frac{1}{6}''$	4 ,,	0·95	45x	0·3
Oil	$\frac{1}{8}''$	3 ,,	1·2	60x	0·2
	$\frac{1}{12}''$	2 ,,	1·2, 1·3, 1·4	90x	0·2–0·12

Eyepieces.

The most common form of eyepiece is the *Huygenian.* It is a negative combination with the field limiting diaphragm between the components.

There are also corrected or *flat-field* eyepieces of better quality and yielding a flatter field. Makers have their own trade names for these.

The **Ramsden** eyepiece is a positive combination with the diaphragm below the lenses. It was formerly preferred for high-power eyepieces but is very little used nowadays except for compensating eyepieces.

Projection eyepieces have a long focus and low magnification. The eyelens is in a focusing tube to accommodate it to the specific image distance used for projection. It has a very small field, but gives an extremely sharp image. It can also be used for photographic work.

Compensating eyepieces are especially designed for use with apochromatic objectives and are over-corrected to compensate for the slight difference in the magnified images of different colours. They may be of the Huygenian or Ramsden type, according to the magnifying power.

Standard eyepieces are as follows (Beck):

Focal length	Magnification
42 mm.	6x
25 ,,	10x
17 ,,	15x
Compensating eyepieces	
45 mm.	6x
30 ,,	8x
22 ,,	11x
15 ,,	17x
10 ,,	25x
5 ,,	50x } for testing
2·5 ,,	100x } purposes only.

Huygenian eyepieces consist of two plano-convex lenses, one at each end of a tube, with a diaphragm between them. It is the standard eyepiece for use with achromats, but it is not the best for photography. The lower of the two lenses is called the field lens, because it increases the size of the field, while the upper or eyelens does the magnifying.

The two lenses are together achromatic and give a fairly flat field for visual purposes, but for photography, where the microscope has to be re-focused in such a way that a real image is formed behind the eyepiece instead of a virtual image projected in front of the observer's eye, the results are not so satisfactory.

Compensating eyepieces, though specially corrected to work with apochromatic objectives, are best for use with achromats, when of a higher power than 15x.

The best eyepieces to use for general work are those of the lowest powers, 5x, or 6x, and 10x, or 12x. The eyepoint is large with low power eyepieces, and specks of dust on the surface of the eye or in any part of the instrument do not readily show. It is, however, a great convenience to be able to slip in a high-power eyepiece in order to increase the overall magnification without altering the adjustments of the instrument (with the possible exception of a slight readjustment of the focus).

CHAPTER V

SELECTING THE RIGHT EQUIPMENT

IT is waste of money and effort to purchase an instrument far beyond your probable requirements, when a simpler equipment will do all that is required just as efficiently and much more easily, but it is a great mistake to attempt difficult work with an indifferent instrument.

The various components must of course bear some relation to each other. It is useless to have an elaborate stand for use with low-power lenses only, and it is equally useless to purchase a wide aperture apochromat of high power without a correspondingly high-class illuminator, or to use such components on a cheap stand designed solely for low-power work.

While it is undoubtedly preferable, at least for the beginner, to purchase a modern instrument from one of the leading makers, there are nevertheless many satisfactory second-hand microscopes for sale, and this aspect of the problem will therefore be discussed first. Cost may be an important consideration, but even second-hand instruments are expensive, if they are really good. A poor instrument is always a disappointment, and an endless source of trouble and discouragement.

SECOND-HAND INSTRUMENTS

It is a great mistake to buy a second-hand instrument merely because it is cheap, or because it has elaborate and impressive fittings and a great number of accessories. The former may be worthless and the greater part of the latter obsolete and useless. An instrument may look sound, but only the expert can really tell whether it is in good working order, or not. Avoid all instruments, however impressive, with tilting stages or unorthodox swinging substage fittings; they were designed for "oblique light", and such devices are entirely useless and long obsolete.

The only safe procedure, when contemplating the purchase of a second-hand microscope, is to ask an expert for assistance and advice. These are always willingly given. If this is impossible the following considerations will be found useful.

There is no particular difficulty if the instrument, though

second-hand, is relatively modern. It can then be obtained from one of the leading makers who usually carry a stock of such instruments which have been reconditioned, and are sold under guarantee. If the instrument is of an older type and purchased privately, or from any dealer other than a leading manufacturer, great caution must be exercised.

Here, the difficulty is twofold. In the first place, the variety of instruments and accessories offered for sale is bewildering. The designs differ widely and many are of non-standard sizes. Some of them may date back to the second half of the last century; some bear maker's names, while others do not.

In the second place, a great deal depends, as might be expected, on the past history of the instrument and its optical components. It may have been roughly handled, exposed to acid fumes, allowed to oxidise, or even dropped.

The lenses may be badly scratched, or the cement between them may have developed flaws. If a high-power lens has been dropped, or inadvertently driven down against the object slide, it may be damaged internally or slightly out of alignment, and this can only be discovered by optical tests which may be very misleading unless they are carried out by an expert.

Some of these older instruments are in a perfect state of preservation and microscopes by Ross, Powell and Lealand, Smith and Beck, Crouch, Collins, and Browning, for instance, may prove to be excellent instruments for certain purposes.

Powell and Lealand.—Instruments by Ross or Powell and Lealand, particularly their first-class stands, are outstanding. The workmanship has never been surpassed, or probably even equalled, and if they are in good condition they will do everything that a modern instrument will do and even more, because they are more adaptable.

While such instruments are provided as a general rule with the standard thread and can therefore be used with any standard object glass, it should be noted that all modern objectives are computed for use on the short, or 160-mm. tube, and cannot therefore be used on the older 10-inch tube.

Ross.—Many Ross instruments were provided with a rather short bodytube fitted with a relatively long drawtube, so that the tubelength can be adjusted to 160 mm. in some cases.

Failing this, it is a comparatively simple matter to cut down the existing tube.

Alternatively, some of these instruments are equipped with two bodies, a 10-inch monocular body complete with drawtube, and a 10-inch binocular body of the Wenham type. Since the latter can be used for monocular work by slipping the prism out, the extra monocular body is redundant and can be cut down to 160 mm.

I use a Ross No. 1 stand arranged in this way and have found that even with such a highly critical combination as a Zeiss 2 mm. oil-immersion apochromat of 1·4 *NA*, and Watson's 1·3 *NA* Holos condenser, it gives entirely satisfactory results, better, in fact, than those obtained with the modern Continental stand also in my possession.

The result, of course, is an instrument of extreme adaptability. It can be used with any object glass, whether corrected for use on a long, or on a short tube. The older eyepieces can be used, and they have many advantages; they have a wider field than modern models and are more comfortable to work with because the exit pupil is somewhat larger and the eyepoint farther away from the eyelens. An adapter slips into the tube to take modern eyepieces of the compensating type.

The instrument combines the advantages of monocular and binocular vision. The substage illuminator, of excellent performance and design, has a standard thread and will take any low-power objective or any modern illuminator. It can also take any of the older accessories such as the dark-field paraboloids, or polarising prisms of very large size, a most useful feature for some classes of work. The weight and size of the instrument ensure great stability at any inclination, and owing to the really superb workmanship, the adjustments are every bit as good as their modern counterparts. This applies particularly to the fine adjustment which is extremely sensitive and remarkably smooth. The stage is very roomy and admirably designed, and the range of the coarse adjustment such that the lowest power lens can be comfortably focused on a fairly large object such as a test tube or petri dish with ample room to spare. Many modern instruments are sadly lacking in this respect.

Wenham Binocular.—These are exceptional instruments, however, and generally speaking the older types should be discarded except for low-power work, such as nature study or botany for instance, where the binocular types will be found of great value. The Wenham binocular is unsurpassed for this class

of work in which powers higher than $\frac{1}{2}$ inch are seldom needed. For tiny flowers and insects, fungi, mosses, algae, pond-life, and similar low-power subjects, no better instrument exists.

"Society of Arts."—Never buy a stand which does not bear a maker's name. There are a great many "Society of Arts" models among the second-hand instruments offered for sale. The origin of the name is to be found in the prize offered by the Royal Society of Arts, and won in 1859 by Field, of Birmingham, to the manufacturer who could design an outfit to fulfil a given specification within a very modest price limit. The original outfit was widely copied, and these instruments vary enormously in quality. They are best left alone.

Older Optical Components.—The older optical components are often of excellent quality though they do not attain the high standard set by modern lenses.

The eyepieces are excellent, but they vary considerably in size, as do the bodytubes of older instruments. It is therefore a matter of extreme difficulty to find additional eyepieces to fit an instrument. The difference between the various diameters is so small as to make the use of an adapter impossible in most cases.

The older object glasses are often very good, but, with a few exceptions, they are of small numerical aperture, and the reader is now in a position to appreciate the fact that a $\frac{1}{10}$ inch or $\frac{1}{12}$ inch of 1 *NA* or less is not of much value in practice. The numerical aperture, or even the older angular aperture, is very rarely indicated on these lenses and it is sound policy to avoid buying any of the older lenses for powers exceeding $\frac{1}{4}$ inch.

There are of course exceptions. Powell and Lealand have produced a $\frac{1}{12}$ inch and even a $\frac{1}{16}$ inch of very high numerical aperture, and I have a water immersion $\frac{1}{10}$ inch by Swift of very ancient vintage which is nevertheless a lens of outstanding quality.

For reasons which will now be obvious, the $\frac{1}{20}$, $\frac{1}{25}$, and $\frac{1}{50}$ inch lenses are merely museum pieces.

TESTING A SECOND-HAND INSTRUMENT

Once again, consult an expert whenever possible. The tests outlined below are often deceptive and many instruments have been cleverly "doctored". Many instruments will not pass every one of these tests satisfactorily, and may yet be quite suitable for certain classes of work, or as auxiliary stands for mounting,

dissections, and similar low-power requirements. They may make excellent portable instruments for routine tests and preliminary examinations. It is obviously impossible for the novice to pass judgment in such cases. This is where experience tells.

When examining an instrument, the following tests should be carefully carried out. No reputable dealer will object to this.

(1) The thread on the nosepiece must be standard. Ask the dealer for a standard objective and test the fit.

(2) Test the instrument for stability by tilting it sideways and inclining the body until it is horizontal. It should show no tendency to tip over.

(3) Test the adjustments generally for lost motion or backlash and smoothness, particularly the coarse focusing adjustment. Strip it, if possible; it may have been packed up with "optician's grease" to conceal wear. You should be able to focus a $\frac{1}{6}$-inch objective on to an object easily and smoothly by means of the coarse adjustment alone.

(4) Attaching a high-power object glass (at least an $\frac{1}{8}$ inch) and focusing an object on the stage, test the fine focus adjustment for freedom from lost motion (there should be no trace of this), smoothness, and sensitivity. If the fine adjustment is of good design, the milled head can be moved through an appreciable angle before the image deteriorates noticeably; if the adjustment is too coarse, the image will be thrown out of focus suddenly, instead of gradually, as soon as the milled head is moved. The focus should be exactly restored when the milled head is moved back precisely to its original position. Avoid fine adjustments consisting of a milled screw and lever fitted *to the nosepiece*; they are very common, and usually unsatisfactory for anything beyond low-power work.

(5) Test the rigidity of the stage by noting the pressure necessary to put the object out of focus, and whether it returns to its original position when the pressure is removed. Test the bodytube in the same way by exerting slight pressure at the eyepiece end transversely, vertically, and longitudinally.

(6) Extend the drawtube and test its motion, fit, and parallelism.

(7) Examine the focusing and centring adjustments of the substage. Test the alignment of the substage. Once centred, it should not move appreciably out of centre when racked up or down. This is easily done by closing down the substage diaphragm

to a small aperture which can be focused through a 1-inch lens, for instance, and then brought into the centre of the field of view by means of the substage centring screws.

(8) Test the mechanical stage by examining a flat preparation (a thin section is best) and noting whether the centre of the field of view remains in focus when the slide is moved from side to side, or up and down. This is a rather searching test unless the object glass is of low power. It is not an essential point, unless high-power work is contemplated, provided that the stage and mechanism are really firm, move smoothly, and have no backlash. It is far better to do without a mechanical stage than to work with an imperfect one.

(9) There must be no flexure of the limb or tube in any direction, with the coarse focusing adjustment fully extended, and no trace of play or looseness anywhere.

(10) The mirror should have both a plane and a concave face. If there is only one face, it should be plane. The mirror should be clear and free from spots and flaws. This is a comparatively minor point, because mirrors can be easily resilvered.

(11) Examine any object glasses supplied with the instrument very carefully. Remember that whatever their magnifying power, as indicated by the focal length, it is the resolution that matters, and the older lenses are almost always of low aperture. Their general appearance is a good guide to their past history. The metal mounts should not be grossly discoloured, stained, or scratched. With the help of a 10x hand lens, examine the front lens for polish and scratches. With a low-power lens, it is often possible to examine the back lens in the same way. Otherwise, and for the higher powers, hold the objective up to the light and examine the back lens through the front lens. The whole appearance should be clear, colourless, and luminous. Look through the lens at an acutely oblique angle; this may show up scratches on the back lens, or flaws and minute bubbles in the cement.

If possible, have a few test slides available with which you are familiar. Examine them with each object glass, to assess its quality and performance. The proboscis of a fly is an excellent test for any lens up to $\frac{1}{2}$ inch inclusive. *Pleurosigma angulatum* is a good test for a $\frac{1}{4}$ inch, $\frac{1}{6}$ inch, or $\frac{1}{8}$ inch, and *Surirella gemma* or even *Amphipleura pellucida* for the highest powers. You cannot, however, gauge the performance of a lens in this way unless you are sufficiently experienced, and know what to expect.

When buying a second-hand object glass separately always insist on taking it on approval, and test it at home, under familiar conditions. Make sure that all objectives are intended for use on the particular tubelength of the instrument you are buying (10 inch or 160 mm.). The sets supplied with second-hand instruments are often "made up" from odd objectives which may not all be corrected for the same tubelength. If the instrument has a short body and a drawtube, it can be adapted for use with long tube lenses, but if it has a 10-inch tube, any objectives corrected for use on a short tube will give a poor performance unless they are of low power (not exceeding 1 inch). With highly corrected lenses, such as the apochromats, the right tubelength is essential. This is a difficult point because the proper tubelength is seldom indicated, except for apochromats, and the deterioration of the image may not be appreciated by the novice, and may of course be due to other causes.

(12) The type of substage illuminator or condenser provided is not of such importance because it is almost always possible to fit a modern illuminator to an existing stage either by means of an adapter, or by removing the substage fittings altogether and getting an instrument maker to mount an Abbe illuminator together with its holder in their place.

Generally speaking, the older condensers, if they are of orthodox type, are of very good quality and amply sufficient for most classes of work.

NEW INSTRUMENTS

When buying a new instrument from the makers, no such difficulties arise.

A modern instrument of good quality can safely be assumed to comply with all the conditions outlined above, and the main considerations are then suitability and cost.

The makers are always prepared to give expert advice, and their catalogues are full of information. The different types listed are easily compared and hence related to the purchaser's particular requirements and means.

Most modern instruments (except such as are intended for the most critical research or for very special fields) are so designed that more complex models can be built up from a simple basic type by the subsequent purchase of additional fittings.

This is of great assistance to the student who can first obtain

quite a simple, inexpensive stand and gradually add to it as he becomes more expert and his work demands more elaborate apparatus.

Among the leading manufacturers at the present day are the following firms: Baker, Beck, Cooke Troughton & Simms, Swift, and Watson in England; Bausch & Lomb, and Spencer in America; Leitz, and Zeiss in Germany; and Reichert in Austria. All produce instruments of the very highest quality.

Modern Features.—Modern instruments tend to be much smaller and more compact than the older models. This rather natural reaction against the unwieldy instruments of the last century has been overdone. Some modern instruments are so cramped that they are very inconvenient in use.

The manufacturer's object in producing models of this type is not easy to understand. An instrument of good quality seldom, if ever, requires moving, still less carrying about, so that its size and weight are of no great importance from this point of view, whereas they are valuable features as regards stability, freedom from tremor, and general convenience.

If an instrument is often moved, or carried from place to place, there are many excellent portable types, and it is far better to purchase a second and simpler instrument for this purpose than to sacrifice roominess, stability, and convenience in a misguided attempt to acquire a combination of both types which usually proves unsatisfactory for either purpose.

For these, and other reasons, the latest stands by the best makers show a marked increase in size, and this is an excellent sign.

The long, or 10-inch tube has become obsolete. The bodytube usually has a length of 140 mm. and can be lengthened by means of a drawtube to 200 mm., and in some cases to 260 mm., which is a very desirable feature.

Apart from considerations of size, the short tube is far more sensitive to adjustments made in order to compensate for cover slips of varying thickness. A bodytube of great length must be moved considerably to produce any appreciable effect, so that a much greater range of correction can be obtained with the short tube. It is also possible to further lengthen a short tube, whereas it is impossible to shorten a long one.

Unfortunately the reduction in tubelength has been combined in some cases with a reduction in the diameter of the tube. This

is a mistake, and the adoption of larger diameter tubes on the more elaborate models is a proof that this practice is being gradually superseded. The wide tube is of particular importance for photographic work.

Range of Models.—There exists a wide range of models graded in accordance with the general nature of the purchaser's requirements. The simplest forms are suitable for the naturalist, or for field work. Portable models range from primitive to quite elaborate types. Then come the simpler student's models, strong, inexpensive, and reliable instruments, but without any trimmings. Then come biological, bacteriological, and research instruments, growing more elaborate as the work becomes more critical. Finally come the series of instruments specially designed for petrographic or metallurgical work, the low-power binocular instruments of the Greenough type, used mainly in industry, measuring microscopes, and others.

Some of the more important specialised applications, together with the types of instruments designed for these purposes will be mentioned in a later chapter. The very simple, rather primitive microscopes sold for purposes of nature study or rough work in the field, are little more than compound magnifying lenses and do not properly belong to the subject-matter of this handbook.

We will therefore confine our remarks in this chapter to the more standard instruments in general use which, in fact, cover the whole ground, with the exception of petrology and metallurgy.

We will first deal with the stand, and afterwards with the optical components.

The Stand.—We have seen that most manufacturers supply a simple, basic stand, which can be built up into an instrument possessing every refinement by the addition of extra components.

The differences between the various models are in the stages and substages. They are so designed that all components such as mechanical stages, illuminating condensers, dark ground illuminators, nosepieces, object glass changers, extra object glasses and eyepieces, can be added as occasion arises without the necessity of returning the instrument for fitting.

While this is a very valuable feature, and instruments built up in this way will undoubtedly cover every requirement met with in ordinary general practice, there remain nevertheless certain further features which cannot well be thus incorporated.

It follows that the leading makers offer in addition research

models specifically designed for the most critical work, and combining every possible refinement with extra weight and stability and the highest order of workmanship. These instruments are naturally expensive, and they will not fulfil the conditions for which they are designed unless they are handled by an expert.

The former type of instrument is therefore the one best suited to the needs of the general user, and his main problem will be to decide which components he should buy and their relative degrees of importance.

Assuming therefore that the stand itself is of good general design, firm and stable, and that it is provided, as a matter of course, with a satisfactory coarse focusing adjustment and a responsive and sensitive fine focusing adjustment, without which fundamental features no instrument is worth consideration, what are the next items, in the order of their importance?

A complete answer to this question is difficult because it depends largely on the class of work done, and also on individual preferences. A decision can best be taken after carefully reading through the various chapters on illumination, the instrument in use, specialised applications, and numerical aperture, but the following list would probably meet the requirements of the average user.

(1) Substage illuminator, Aplanatic type.	No serious work can be done without some form of substage condenser. This is really an essential component, and it must be fitted with an iris diaphragm.
(2) Rack and pinion (or similar) substage adjustment.	Essential for medium- and high-power work. The rack and pinion type is the best.
(3) Centring device on substage.	A great convenience. Essential for achromatic illuminators, and for dark-field work.
(4) Mechanical stage.	For general convenience, systematic searching, or measurements.
(5) Achromatic illuminator, or special highly corrected illuminators.	For use with high power achromats, and apochromats generally. Such lenses will not give the best results without them.

(6) Rack and pinion drawtube adjustment.	This is more than a refinement, it is a very real convenience when accurate compensation is required with apochromatic lenses, or high-power achromats. It is very useful for measurements and a safeguard against the drawtube slipping out of position.
(7) Second drawtube.	Enables the tubelength to be extended to 250 mm. so that lenses computed for this distance can be used with correspondingly greater initial magnification.
(8) Fine focusing adjustment to substage illuminator.	This is a refinement, necessary only for the most exacting work. It is certainly convenient for high-power dark-field work, or when using illuminators of very short focal length.
(9) Rotating stage.	Useful for photographic work, otherwise, only for metallurgy, petrography, crystallography, and polarised light.

Optical Components.—Generally speaking, achromats, or at most semi-apochromats, will do all that is required. Apochromats are necessary for very critical work, and for photomicrography.

The ideal, where expense does not matter, is to have a set of achromats for general, routine, and preliminary work, and a set of apochromats for more exacting work.

The next best alternative is a complete set of semi-apochromats.

Failing this, a set of good achromats from any leading maker will give entire satisfaction and the novice, at any rate, should not attempt to use anything else.

The so-called "powers" which make up a set of objectives have changed in recent years, mainly on account of the universal adoption of the 160-mm. tube, and metric focal lengths. The change and corresponding designations for the older and more modern equipments are best shown in the form of a comparative table:

Initial Magnification Diameters	Older powers 10″ tube	Modern standard lenses 160 mm. tube	Additional lenses not included in standard sets
5x	2″	25 mm. 1″	32 mm. $1\frac{1}{3}$″
10x	1″	16 ,, $\frac{2}{3}$″	— —
20x	$\frac{1}{2}$″	8 ,, $\frac{1}{3}$″	— —
25x	$\frac{2}{5}$″	— —	— —
40x	$\frac{1}{4}$″	4 ,, $\frac{1}{6}$″	— —
50x	$\frac{1}{5}$″	— —	3 ,, $\frac{1}{8}$″
80x	$\frac{1}{8}$″	2 ,, $\frac{1}{12}$″	— —
120x	$\frac{1}{12}$″	— —	1·5 ,, $\frac{1}{16}$″

It will be seen that the older standard 10-inch tube set of objectives consisting of 1 inch, $\frac{1}{2}$ inch, $\frac{1}{4}$ inch, $\frac{1}{8}$ inch and $\frac{1}{12}$ inch has been superseded by the more modern set consisting of $\frac{2}{3}$ inch, $\frac{1}{3}$ inch, $\frac{1}{6}$ inch and $\frac{1}{12}$ inch giving, on the 160-mm. tube, the same steps of initial magnification, namely 10x, 20x, 40x, and something of the order of 100x.

The 24-mm. lens, corresponds to the 2-inch, with a magnification on the short tube of approximately 6x.

The most popular combination now listed as standard equipment consists of the following lenses:

1 in.	24 mm.	6x	*NA* 0·15
$\frac{2}{3}$ in.	16 mm.	10x	*NA* 0·28
$\frac{1}{6}$ in.	4 mm.	40x	*NA* 0·65

with 6x and 10x eyepieces, giving overall magnifications of 36, 60, 100, 240, and 400 diameters. This is an excellent combination suitable for any class of general work.

It can be completed by the addition of a 32-mm. lens, and later by the addition of a 2-mm., or $\frac{1}{12}$ inch oil-immersion objective of 1·25 *NA*.

Oil-immersion lenses of 1·30 or 1·40 *NA* are always apochromats and are only necessary for advanced research work and photomicrography. Needless to say, they are very expensive and require correspondingly corrected and expensive illuminators.

Most modern achromats, and all semi-apochromats and apochromats will stand considerable eyepiecing, so that the equipment can be usefully completed by the inclusion of 12x (or 15x) and 20x eyepieces. For the examination of the very finest detail with the best apochromats a 25x eyepiece is often advantageous.

When selecting optical components, the data given in the chart on page 107 will be useful.

While there is no point in an undue amount of overlap, nevertheless it may be worth considering the purchase of intermediate powers which, though they may give practically the same overall magnification, will differ from other lenses in having a rather larger working distance, or a greater depth of focus, or even a more extensive field of view. The advantages can only be obtained at the cost of a reduced numerical aperture, and therefore resolution, but there are circumstances under which their importance may warrant this.

The most important thing for the novice to realise is that there is nothing to be gained by purchasing a large number of unnecessary lenses when a few will serve the purpose. Nor is anything to be gained by the purchase of high-power lenses, particularly of the immersion type, or expensive apochromats of high aperture until considerable experience has been acquired with low- or medium-power achromats. Such lenses are not of the slightest use to the beginner who will be unable to get the best performance out of them, and if he fails to do that, he will get no better image than with a good achromatic lens, and in all probability a worse one.

Finally, when making up a set of optical components, remember that resolution, not magnification, is the criterion. It is the numerical aperture which tells.

The advantages of high numerical aperture must be weighed against such considerations as price, reduced working distance, and depth of focus. Here again the advice of an expert, preferably one familiar with the requirements of the class of work you intend to do, will be of great value. If you are dealing with one of the leading makers, ask for advice. It will be freely given, and reliable.

The following table is intended as a rough, preliminary guide, and no more.

Class	Overall Magnification	Object glass focal length mm.	Initial Magnification 160 mm.	Eyepiece
I	10–100	32, 24, 16	5, 6·5, 10	6x, 10x
II	100–300	16, 8, 4	10, 20, 40	6x, 10x
III	300–500	4, 3, 2	40, 55, 80	6x, 10x
IV	Over 500	4, 3, 2, 1·5	40, 55, 80, 120	6x, 10x, 15x, 20x

Class I. Includes nature study, botany, entomology, minute animal and vegetable organisms (protozoa, lower metazoa, algae, desmids, fungi, mycetozoa, parasitic life, minute aquatic life, etc.). Materials of commerce and industry. Fibres, textiles, papers, pigments, powders, ores, chemicals, drugs, etc. Criminology.

Class II. Finer details under above headings. Larger cells, smaller crystals, histology, pathology, haematology.

Class III. Histology, pathology, bacteriology, cytology.

Class IV. Bacteriology, diatoms, cytology, chromosomes, research.

It may be worth while mentioning here that the once popular $\frac{1}{8}$ inch has rather gone out of fashion. The reason for this is easy to understand. If the $\frac{1}{8}$ inch is a dry lens, it will require the most careful adjustment and will be extremely sensitive to the slightest variation in cover glass thickness or tubelength. It will also have a very short working distance. If it is of the oil-immersion type, it has little or no advantage over the $\frac{1}{12}$ inch.

It is far better to use a $\frac{1}{6}$ inch of high aperture and a $\frac{1}{12}$ inch oil-immersion lens instead.

The older and once popular one-tenth oil-immersion lens has been superseded by the $\frac{1}{12}$ inch which has an initial magnification more in keeping with the numerical aperture expected from a lens of this nature.

A last word of warning. Avoid all composite objectives. They are so made that the front lens can be removed, the remainder forming a combination of lower magnifying power. Often a second lens can once again be removed with a further reduction of the magnification so that the object glass has really three powers combined in a single unit, usually 1 inch, $\frac{1}{2}$ inch, and $\frac{1}{4}$ inch. No computer can possibly design a lens which will be properly corrected in all three cases. Such lenses (which are always of very low *NA*) can be useful on a rough portable stand for preliminary work in the field, but that is all.

ACCESSORIES

The older text-books contain descriptions of a truly formidable number of accessories, most of which are fortunately obsolete. If we exclude those accessories which relate to mounting slides and preparing material for examination, there are not a great many left which deserve serious consideration.

Here again the user can only decide which of them are essential to his work, after reading over the various chapters in which their use and significance are discussed.

They cannot be grouped into a single list, because they differ too widely in purpose.

As a rough guide to their order of importance, they are here divided into four groups.

The first group is fully discussed in Chapter VI.

The third group is covered by Chapter VIII, and the fourth group briefly in Chapter XI. The two devices in the second group have already been described.

Illumination.	(1) Condensing lens. (2) Vertical illuminator. (3) Oil-immersion dark-field illuminator. (4) Davis, or nosepiece diaphragm.
Nosepiece fittings.	(1) Revolving nosepiece. (2) Objective changers.
Measuring devices.	(1) Micrometer eyepiece. (2) Focusing micrometer eyepiece. (3) Filar micrometer eyepiece.
Special devices.	(1) Polarisers. (2) Binocular attachments. (3) Spectroscopic attachment. (4) Combined illuminator units.

CHAPTER VI

ILLUMINATION

THE microscope has certain definite limitations, but in practice the lighting system proves to be the limiting factor rather than the microscope. Hence, every care must be taken to secure proper, or "critical", illumination.

There is nothing complicated or difficult about this once the fundamental principles have been understood, and the correct method used in each case to suit the particular object under examination, and the initial magnification required.

In the majority of cases where expensive equipment has been carefully selected, little attention is given to the lamp. However perfect the outfit, success will always depend on proper illumination, and no instrument or lens can be expected to give anything approaching its true performance unless this point has received careful consideration. Nearly every failure to obtain satisfactory results may be set down to improperly adjusted illumination; there is very little else that can go wrong. The microscope, particularly in its modern form, is really a very simple instrument, and illumination is the only adjustment which calls for skill, judgment, and experience. Therefore the secret of successful microscopy, whether visual or photographic, is correct illumination.

Skill and experience can only be acquired, while judgment depends partly on experience, and partly on a thorough grasp of the principles involved. The object of this chapter is to give the clearest possible explanation of these principles.

Unfortunately illumination is too briefly discussed in all but the most advanced text-books, and the relevant information too often scattered under different headings.

It should first be made quite plain that artificial lighting is essential. The window and white cloud arrangement is totally inadequate. Artificial lighting is required to produce an image of the light source at once precise, constant, and undiffused. This, when sharply focused on the subject, constitutes critical illumination. According to the modern theory of microscopic vision, conditions are ideal when the object under examination is self-luminous or, in other words, when every point of the object acts

as a self-luminous source of light from the point of view of the optical components (other than the illuminator).

A near approximation to this condition is obtained when an image of the light source is accurately focused on the plane of the object, the light rays crossing at points within the plane and thereafter diverging as though emitted from the object itself. Hence the importance of focusing the light-source accurately and sharply on the object plane, and of using properly corrected illuminators for the purpose.

This is the basis of critical illumination.

ILLUMINATING SYSTEM

This includes the lamp, and the illuminator proper, concave mirror, bull's eye, vertical illuminator, dark-ground illuminator, or condenser, as the case may be (Fig. 19).

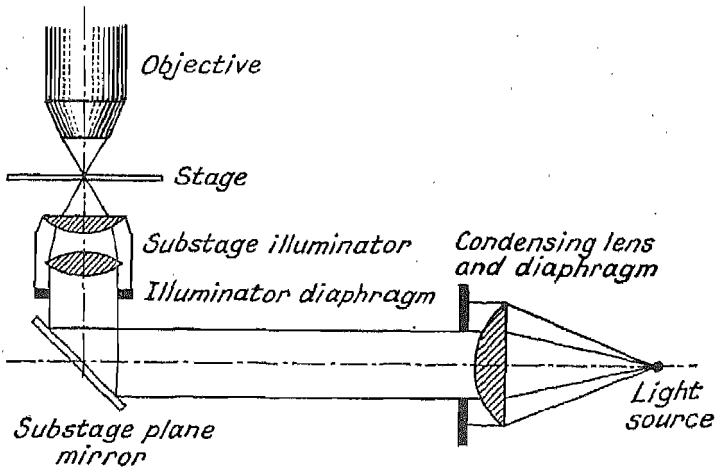


FIG. 19.

Methods of Illumination.—Various methods of illumination are used according to the nature of the object examined. In the majority of cases the object is transparent and can be illuminated from below, the light rays passing through the object into the object glass.

Transmitted Illumination.—This is known as transmitted, or direct illumination. The object and its structural detail will only be visible if:

(*a*) They are naturally coloured; *or*
(*b*) They are artificially stained; *or*
(*c*) If colourless, their refractive indices are greater or lower than that of the medium in which they are mounted. The difference between the values of the refractive indices of medium and object is known as the index of visibility.

It is not always possible to stain objects, nor is it always possible to use a mounting medium of high refractive index.[1] An obvious example of this is the examination of living bacteria or pond life specimens, many of which are almost invisible when examined by transmitted light. Here the medium must be that in which the organisms live.

Dark-Field Illumination—In such cases dark-field illumination can be used. The object itself is shown as a brightly illuminated body against an almost perfectly dark background. There are various means of doing this which will be described in detail later. The illumination is only transmitted inasmuch as it is supplied by an illuminator beneath the stage, but the only rays which are allowed to enter the object glass are those reflected from the specimen itself.

Dark-field illumination is thus really a reflection method.

Reflected Illumination.—When examining opaque specimens, such as the larger insects, or metallurgical specimens, for example, the light must be thrown on to the specimen from above. The light entering the object glass is thus reflected from the specimen itself.

These are the three main methods of illumination and they cover every possible need.

Oblique Illumination.—There is a further method of examining objects, particularly when it is required to reveal very fine symmetrically recurrent structures such as the markings on diatoms. This is usually a transmitted light method in which a narrow beam of extreme obliquity is thrown upon the object from underneath, but oblique reflected illumination is also used. This method and the resulting images have been and still are the subject of much controversy. The problem is very closely related to the theory of diffraction spectra and microscopic vision, and the method is generally regarded with justifiable suspicion.

[1] A refractive index lower than that of glass cannot be used without seriously affecting the numerical aperture of the object glass in the case of lenses of high aperture and oil-immersion.

GENERAL PLAN OF THIS CHAPTER

(1) The functions of the various components of the illuminating train, lamp, condensing lens, substage illuminator, and diaphragms will first be described.

(2) Each of the methods outlined above will be discussed in turn, and simple instructions will be given to enable the student to carry out the adjustments quickly and correctly. It is useless to give such instructions without first explaining the underlying principles. To do a thing correctly, you must first know exactly what you are trying to do.

(3) A schedule, in tabular form, is appended for each method. This is a comparative table setting out the principles, applications, advantages, and disadvantages in each case.

THE ILLUMINATING TRAIN

Distance from Light-Source to Instrument.—The total distance from light-source to object depends, to a certain extent, on the type of substage illuminator in use. As a general rule it should be of the order of 10 or 12 inches. When the beam is reflected by a substage mirror upwards into the instrument the total distance is of course the sum of the distances from lamp to mirror and from mirror to specimen.

The Lamp.—It is very little use spending a great deal of money on a really good instrument and priding oneself on the possession of a high-power lens of large aperture if one has spent but a small fraction of that sum on one of the many crude devices misleadingly offered for sale as microscope lamps.

The number of lamps described in some text-books is apt to be confusing and a great many can be ruled out as obsolete or inconvenient. Here are a few satisfactory and representative types:

General Purpose Lamp for Low Powers and Routine Work.—For low powers, and for a great deal of routine examination with medium powers, an ordinary "opal" (not pearl) electric bulb is entirely satisfactory. The light, however, is too diffused for critical work with high powers. Alternatively, an ordinary bulb can be used with a fine grain ground glass screen in front of it.

The bulb should be enclosed in a properly ventilated and reasonably light-tight housing provided with a circular aperture fitted with an iris diaphragm (Fig. 20).

A condensing lens can be incorporated with the lamp or a condensing lens mounted on a separate adjustable stand can be used. Some form of filter holder should also be available.

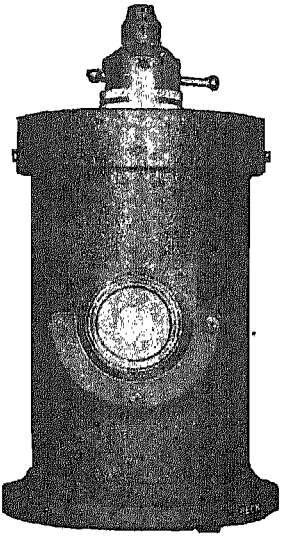


Fig. 20.—Plain lamp in simple housing with diaphragm and filter-holdor (Beck).

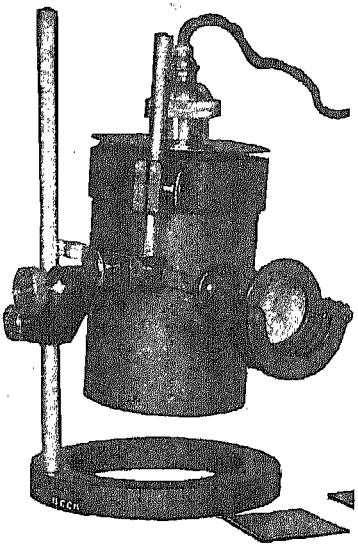


Fig. 21.—"Pointolite" lamp (Beck).

"Pointolite" for all Purposes.—For truly critical work with medium and high powers, and for photomicrography, the ideal lamp is probably the "Pointolite". The light is emitted by a small incandescent sphere about the size of a peppercorn. It can be used with direct or alternating current, and is made in two sizes (100–150 and 30 candle-power). This lamp most nearly approximates to a point source. It is fitted with a condensing lens, an iris diaphragm, and a filter holder (Fig. 21).

Rod Lamp for all but Highly Critical Work.—A very suitable lamp, where highly critical results are not essential, is manufactured by C. Baker. It uses a 6-volt 4·5-ampere bulb fed from a small transformer. An optical glass rod is fitted in front of the lamp housing from which it protrudes, the other end being pushed up close to the surface of the bulb. One end of the rod is polished,

the other finely ground. It is from this latter protruding end that the illumination is obtained, the light travelling up the inside of the rod through a series of total reflections.

The front end of the rod carries a filter holder which can be replaced by a rectangle prism for reflecting the light direct into the substage illuminator or dark-field illuminator. The prism can also be used for illuminating opaque specimens on top of the stage (Fig. 22).

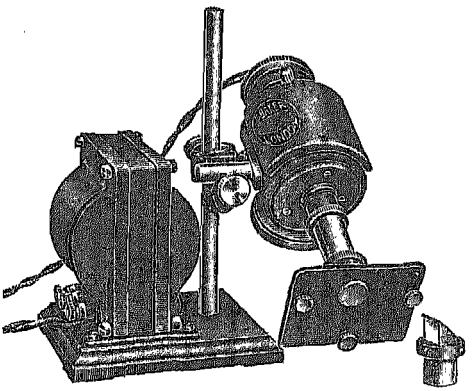


FIG. 22.—Optical glass rod and prism lamp (C. Baker).

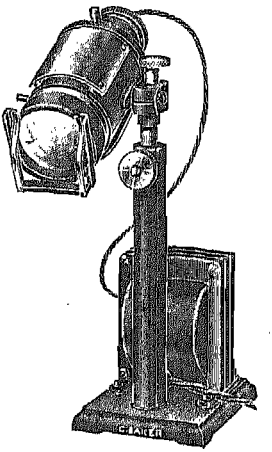


FIG. 23.—Research lamp with large focusing condenser and diaphragm (C. Baker).

Baker 6 V. Bulb Lamp for all Purposes.—For more critical work and photomicrography the same firm supply a similar lamp, but without the glass rod device. The front of the lamp housing is fitted instead with a large adjustable condensing lens, a filter holder, and an iris diaphragm. This lamp is very satisfactory and the bulb can be centred within the housing (a very valuable feature). It would be greatly improved, however, if provision were made for the introduction of a finely ground glass screen just in front of the bulb in order to break up the image of the filament coil which is sometimes obtrusive, particularly when the lamp is being used for photomicrography (Fig. 23).

Lamp Stand.—All lamps, of whatever type, should be arranged to slide up or down a vertical support, so that the height can be adjusted within wide limits, and it should also be possible to tilt the optical axis out of the horizontal.

The Condensing Lens.—The condensing lens, when used, is placed between the lamp and the mirror (except when illuminating opaque objects), and its position will depend on what it is required to do. It can either concentrate the light (convergent beam) or it can be used to obtain an approximately parallel beam. It has three main functions.

For Intense Light

(*a*) It can be used to concentrate an intense spot of light on to the specimen either from above, or from below. This amounts to the formation of a greatly reduced image of the light-source, with a corresponding increase in its intensity.

Enlarging Image of Light-Source

(*b*) It can, on the contrary, be used to form a magnified image of the light-source, with a corresponding decrease in its intensity. This is important because many of the more intense sources closely approximate to a point and may not be large enough to fill the back lens of the illuminator or the field of view. Since the image of the light-source must be focused in the object plane for critical illumination, it may be very disturbing or result in an unevenly illuminated field. If the image of the light-source is magnified, a small homogeneous portion of the light source may be sufficiently enlarged to cover the field evenly and completely.

Parallel Rays

(*c*) It can be used to produce a beam of parallel rays of light, in which case it is so placed that the light-source lies at its principal focus.

Achromatic substage illuminators of high numerical aperture are computed for use with approximately parallel rays, and will only give the best results under these conditions. Perfect parallelism cannot be realised in practice because the light-source is never a true point, but a good approximation can nevertheless be obtained.

Parallel light is an essential requirement for dark-field illumination.

When paralleling rays with the condensing lens,[1] the intensity

[1] When paralleling rays with the condensing lens, the image of the light-source formed by the lens (some image will always be formed even when the rays are parallel) should be received on a card. When the card is moved nearer to, or farther away, from the light-source, the size of the image should not alter appreciably, if the rays are parallel. If the image grows *larger* when the card is moved *away* from the source, the condensing lens should be brought *nearer* to the lamp, and vice-versa.

of the light is considerably reduced, but this is compensated, to a large extent, by the exceptional brilliancy of the image produced by illuminators of high numerical aperture.

True Source of Light.—It has already been stated, in anticipation of a complete definition, that one of the requirements for critical illumination is that the light-source should be sharply focused on to the plane of the object, a result obtained by adjusting, or focusing, the substage illuminator. In other words, when the specimen is focused in the usual way through the instrument, a sharp image of the light-source should be seen simultaneously and superimposed upon the image of the specimen. There appears to be some uncertainty as to what constitutes the source of light.

(1) When the source of light is an opal incandescent lamp or a plain lamp behind a ground glass screen, or again the ground end of the optical rod in the Baker lamp, then the surface of the bulb in the first case, and the ground glass surfaces in the second and third cases constitute the light-source which it is required to focus.

The filament itself will usually be sufficiently masked by diffusion (in addition to being out of focus) to be practically invisible and an evenly illuminated field of reasonable intensity and ample area will result. This is precisely what is required for low power or non-critical work.

(2) When using a light-source which closely approximates to a point, or where the pattern of the filament is sharp and clear, and not masked or broken up by diffusion, matters are not quite so simple.

If no condensing lens is used, there is no doubt about the source of light to be focused though the result will probably be a poorly illuminated field.

(3) If a condensing lens is used to enlarge (or reduce) the image of the light-source with an object glass of average power, then the image of a portion of the filament will be focused in the field of view. In other words, the enlarged (or reduced) image of the filament has become the light-source. It is possible, however, in this case to focus, as an alternative, the iris diaphragm immediately in front of the condensing lens, and a more evenly illuminated field will probably result though the illumination will be less intense.

(4) As soon as the condensing lens is arranged to give a beam of parallel rays, the lamp filament ceases to be the source of

light as far as the microscope is concerned, and there is no true substituted image, so that the condensing lens itself becomes a light-source emitting parallel rays. This means that the substage illuminator sees no difference between this and an evenly illuminated disc of light at an infinite distance. Under these conditions the condensing lens itself or, more conveniently, the aperture of the diaphragm immediately in front of it should be focused.

Ground Glass Diffusors.—Ground glass screens cannot be used with condensing lenses without certain precautions.

The introduction of a diffusor between the condensing lens and the substage illuminator will obviously upset the whole arrangement. It is less obvious, but equally true, that a diffusor between the light-source and the condensing lens will have a similar effect. The ground glass diffusor now becomes the light-source, so far as the condensing lens is concerned, and it is no longer at the principal focus, nor does it approximate to a point, nor is the light coherent. A very fine ground glass diffusor can be used to diffuse the light or, more correctly, further to break up the filament image, but it must be placed as close to the filament and as far from the condensing lens as possible. A good approximation to critical lighting together with a very evenly illuminated field will result.

Condensing Lens Diaphragm.—The condensing lens diaphragm acts in different ways under different conditions.

Its main function is to control the size, nature, and intensity of the beam of light thrown on to the back lens of the substage illuminator.

(1) If the condensing lens is used to concentrate an intense beam of light on to a small area, closing the diaphragm will reduce the intensity of the light without, at first, appreciably reducing the diameter of the illuminated area.

(2) If the condensing lens is used to produce an enlarged image of the light-source, closing the diaphragm will reduce both the intensity and the illuminated area simultaneously.

(3) If the condensing lens is used to produce a beam of parallel rays, closing the diaphragm will immediately reduce the diameter of the illuminated field without, at first, appreciably reducing its intensity.

The Substage Illuminator Diaphragm.—The sole function of this diaphragm is to ensure that sufficient light reaches the

back lens of the object glass to fill it evenly and completely with light, *and no more.*

If the back lens is not completely filled, the numerical aperture is reduced, and hence the resolving power. If the light is in excess of this, the image will be fogged by "glare".[1]

If the diaphragm is closed down to any considerable extent under the mistaken idea that it improves definition or increases the depth of focus, diffraction phenomena will appear. Under these conditions fine detail will almost certainly vanish, and the image seen may be extremely misleading. It may bear little or no relation to the actual structure of the specimen.

Substage Illuminator.—The substage illuminator has already been dealt with in a general way when describing the component parts of the instrument and their functions.

The most generally useful substage illuminator is the ordinary Abbe condenser, and this is sufficient for most purposes. The numerical aperture is usually equal to unity. This may be increased slightly if it is oiled to the slide.

(1) The same condenser or substage illuminator cannot be used with all object glasses. In the first place its numerical aperture must be at least equal to that of the object glass in use, or it will not produce a light cone of sufficient diameter to fill the object glass lens completely. If the numerical aperture of the object glass is A and that of the condenser B, A being greater than B, the effective numerical aperture of the combination will be:

$$\frac{A+B}{2}$$

that is, lower than that of the object glass, but higher than that of the illuminator.

(2) The higher the numerical aperture of the illuminator, the shorter its focal length, and the smaller the area of the image produced in the field of view.[2]

[1] Some microscopists are under the impression that if the field of view is not full of light, and if only a small disc in the centre of the field is illuminated, the objective is not completely filled with light. This is not necessarily so. When using a well-corrected substage illuminator, the back lens of the objective will be completely filled with light, however small the illuminated area of the field of view. Restricting the illuminated area reduces glare when examining very fine structure.

[2] The substage illuminator is required to supply a large quantity of light or, in other words, an extremely brilliant image, much more brilliant than is required for an object glass of equivalent aperture. The brilliance of the image, as every photographer knows, is inversely proportional to the ratio of the focal length and the semi-diameter of the lens. The diameter of the back lens of the illuminator, while it can be considerably larger than that of an object glass, is nevertheless limited by constructional considerations, so that greater brilliancy can only be obtained by reducing the focal length.

An illuminator of high numerical aperture, therefore, may completely fail to cover the field of view if used with a low-power objective, and this, of course, cannot be corrected by any manipulation of the condensing lens since this will only increase the size of the light-source image within the circumscribed area illuminated.

The cure for this is to use an illuminator of smaller aperture, or, when this is possible, to remove the top lens of the illuminator, which produces the same result.

(3) For highly critical work, achromatic or semi-apochromatic illuminators are used, such as Watson's Holos condenser which has a numerical aperture of 1·3 for the standard model and 1·37 for the special model.

When using illuminators of this type, which are always oiled to the slide, it is essential to use parallel light.

A short focal length is essential in order to maintain oil contact with thin slides, but if the focal length is too short, none but the thinnest slides can be used and there is very little scope for adjustment.[1]

TRANSMITTED ILLUMINATION

Low-Power Work.—For use with the lowest powers, it is generally sufficient so to adjust the substage mirror and light-source (no condensing lens or substage illuminator being required) that the field of view is amply and evenly illuminated. It is best to use the concave face of the mirror, though the plane face may sometimes give better results. A sheet of pure white matt paper lightly gummed to the plane face of the mirror will produce excellent illumination. It is essential that the centre of the mirror should at all times lie on the optical axis of the instrument and that the beam of light should be symmetrical about that axis.

It is an advantage to use a light-source of fairly large area, but if the light-source available is too small, an enlarged image can readily be obtained by placing a condensing lens between the lamp and the mirror and adjusting its position until illumination is satisfactory.

[1] Convergent light requires raising, divergent light lowering the condenser. If the slide is so thick that the illuminator cannot be focused, a slightly divergent beam may be tried. If, on the other hand, the focal length is so great or the slide so thin that oil contact cannot be maintained, a slightly convergent beam may cure the trouble. These are, however, at best expedients.

With rather higher powers, a substage illuminator should always be used. When this is in position the *plane* side of the mirror only should be used, because the sole function of the mirror is now to reflect the beam of light unaltered from the lamp into the back lens of the illuminator.

Critical Illumination.—For anything but the lowest powers or routine work with average powers, critical illumination must be used. Otherwise, the full resolving power of the object glass is not being utilised and there is no justification whatsoever for using the higher power.

The illumination is said to be critical when the following conditions are simultaneously fulfilled:

(1) The illuminating beam should be symmetrically disposed about the optical axis of the instrument.

(2) The image of the light-source should be seen in sharp focus in the plane of the object when the object is itself focused through the instrument.

(3) The field of view should be completely and evenly illuminated.

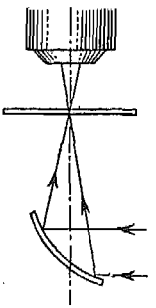
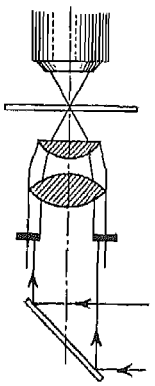
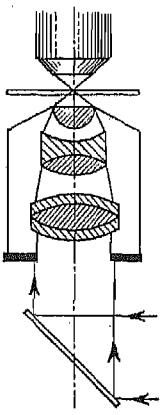
(4) The back lens of the object glass should be entirely and evenly filled with light, and no more.

The first point needs little amplification. The eyepieces and object glass must be assumed to be correctly aligned, but, because object glasses are not all exactly centred in their mounts, it will generally be necessary to centre the substage illuminator for each objective. Provision is made for this on properly designed instruments.

The second and third points are often difficult to conciliate, since a sharply focused lamp filament is incompatible with an evenly illuminated field. Much can be done to overcome this difficulty by a judicious use of the condensing lens, and when parallel light is used, the difficulty does not arise to the same extent. Moreover, modern practice tends towards greater latitude on the second condition and the substage illuminator can be adjusted slightly above its focal point in order to obtain more even illumination. This is in effect what occurs when the so-called Kohler method is used. We will return to this point later.

The fourth point is extremely important, the reasons for this have already been explained when dealing with the substage illuminator diaphragm.

Schedule I. TRANSMITTED ILLUMINATION

System.	Diagram.	Uses.	Advantages.	Disadvantages.
Concave mirror. No substage illuminator. Mirror to stage on lamp distance adjusted to concentrate light on the specimen. See page 79.		Lowest powers.	The large field of view can be fully and evenly illuminated, or a smaller portion of the field can be brightly illuminated.	Only suitable for use with the lowest powers.
Simple illuminator or Abbe aplanatic. Image of source focused on plane of object. Diaphragm restricts light cone to fill back lens of objective. See page 82.		Medium powers. Simple Photomicrography.	Critical lighting. Will illuminate a fairly large field of view. Condensing lens (not shown) can be used to adjust conditions.	Aplanatic cone will not exceed NA=1, and will only reach this value with the best condensers.
Achromatic and Holoscopic illuminators. Parallel light or Köhler. See page 83.		Highest powers. Oil immersion. Critical photomicrography.	Critical lighting. Highest resolution. Perfect definition. High NA to fill high aperture objective. Cone of light aplanatic over full aperture which may be as high as NA=1·40.	Oil immersion for both objective and illuminator. Short focus. Slide must be of correct thickness. With methods used, illumination may be less intense, but this is compensated to a large extent by the very bright image obtainable with these illuminators.

Let us see which of the components of the illuminating train affect the four conditions of critical illumination.

Conditions	*Adjustments*
(1) The illuminating beam should be symmetrically disposed about the optical axis of the instrument.	Aligning lamp condensing lens and mirror. Centring substage illuminator.
(2) The image of the light-source should be seen in sharp focus in the plane of the object when the object is itself focused through the instrument.	Adjusting the position of the substage illuminator. In other words, focusing the illuminator accurately.
(3) The field of view should be completely and evenly illuminated.	Adjusting the position of the condensing lens with corresponding readjustments of the substage illuminator. Adjusting the condensing lens diaphragm.
(4) The back lens of the object glass should be entirely filled with light, and no more.	Adjusting the substage illuminator diaphragm.

PRACTICAL ADJUSTMENTS

(1) **Alignment and Centring**—Remove the eyepiece, object glass, and illuminator. Close the condensing lens diaphragm down to a small aperture, and look down the tube. If the condensing lens and light-source have been approximately aligned to start with it is a simple matter to adjust the mirror so as to throw a beam of light straight up the centre of the tube.

Replace the illuminator and open the condensing lens diaphragm. Close down the illuminator diaphragm to a small aperture and rack up the illuminator till the front lens is level with the stage. With an inch object glass and a low-power eyepiece, focus the illuminator diaphragm in the field of view. Bring the small iris aperture into the centre of the field by means of the adjusting screws. The substage illuminator is now centred. It may require slight readjustment when another object glass is substituted.

(2) **Focusing the Illuminator.**—With the object glass you intend to use in place (unless it is of high power in which case a preliminary adjustment should be made with a $\frac{1}{6}$-inch at most) bring the illuminator into the approximate position and focus the object on the slide.

Place the condensing lens between the light-source and the mirror in such a position that the back lens of the illuminator is completely filled with light, but not much more.

Now adjust the substage illuminator to bring the image of the light-source into sharp focus in the field of view.

(3) **Even Illumination.**—Examine the field of view and, after removing the eyepiece, the back lens of the object glass. Both should be fully and evenly illuminated. If this is not the case, or if the image of the light source is obtrusive, adjust the position of the condensing lens (bringing the image back into sharp focus each time). With a little practice a position will be found in which the field is evenly illuminated.

If, at any time, the light is too bright for comfort, insert one or more neutral tint filters into the filter holder.

(4) **Filling the Back Lens of the Object Glass, and no more.**—Remove the eyepiece, look down the tube at the back lens which should now be evenly illuminated, and close the substage illuminator diaphragm gradually until its edge appears as a concentric circle just within the outer circumference of the back lens. A cleaner image may sometimes be obtained if the diaphragm is closed down a little beyond this, but under no circumstances should this exceed the outer third of the back lens diameter.

PARALLEL RAYS

When using parallel rays, the matter is even simpler because no further adjustment of the condensing lens is required or indeed possible.

It will be remembered that it is the condensing lens diaphragm which must now be focused in the field of view. An evenly illuminated field is much more readily obtained, but the intensity of the illumination is considerably reduced.

Köhler Illumination.—The position of the condensing lens is now slightly altered so that an image *of the light-source* is thrown on to the substage illuminator diaphragm (which should be closed temporarily to receive the image).

The substage illuminator is then readjusted so as to bring *the condensing lens diaphragm* into sharp focus.

It is found that under these conditions an image of the light-source is formed in or near the back focal plane of the object glass.

This form of illumination is best suited to photomicrography, and when the above adjustments have been made it will be found that the condensing lens diaphragm is sharply focused on the camera screen.

Schedule I gives a comparative summary of the different systems of transmitted illumination.

DARK-FIELD ILLUMINATION

Applications.—Visibility depends on contrast between the object and its background. Contrast is sometimes sufficient, or it can be obtained or increased by staining. It can also be accentuated by using mounting mediums of high refractive index, such as monobromide of naphthalene or artificial realgar.

These methods cannot always be applied. The preparation may be already mounted, or the organisms may be alive. Killing and staining produce profound changes in certain organisms. Dark-field illumination can be used with advantage. It is now mainly used for the examination of bacteria, and particularly for counts, an application where contrast and visibility are of great assistance.

The chief value of this method thus lies in the increase in visibility obtained, but no actual increase in resolving power as regards the separation of detail will result. A drawback is that it may produce confusing diffraction effects round the image, thus increasing the difficulty of correct interpretation.

Principle.—In order to obtain dark-field illumination the light which illuminates the object must not enter the object glass direct. The only light admitted to the object glass must be that which has been scattered or reflected by the object itself. This is immediately apparent if we compare the conditions for critical transmitted illumination and dark-field illumination set out in detail in Fig. 24.

The common feature of the methods described (except the first) is to supply a very wide cone of light, in excess of that required to fill the object glass, and then to stop out that portion of the

cone which would normally enter the front lens of the object glass direct. The oblique rays which remain, and which are emitted at such an angle that they miss the object glass altogether, are

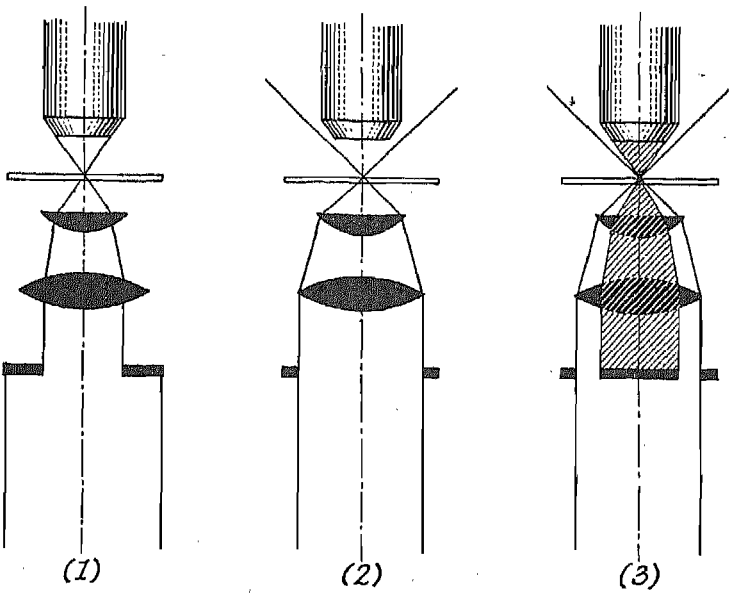


FIG. 24.—The principles of critical transmitted, and dark-field illuminations compared.

(1) This, when in addition the light source is focused on the object plane, is critical illumination. The light cone just fills the objective and no more. The illuminator diaphragm is adjusted to ensure this.
(2) The illuminator diaphragm is fully open, and the light cone greatly in excess of that required to fill the objective. Oblique rays which *cannot enter the objective serve no useful purpose as transmitted* light, but they will be reflected and refracted back into the lens to a certain extent, by the specimen. This results in glare, and a foggy, ill-defined image. Under such conditions we are, in effect, trying to examine the specimen by transmitted and reflected light simultaneously, with poor results.
(3) If we now insert a dark stop, we entirely cut off the light cone which would otherwise just fill the objective lens. The oblique rays, which can only enter the lens after reflection or refraction from the specimen itself, remain unaffected. This is the correct adjustment for dark-field illumination and the specimen, now the sole visible source of light from the observer's point of view, will stand out brightly illuminated against a perfectly dark background.

nevertheless scattered and reflected by the object, and it is this scattered and reflected light which enters the object glass to form a brilliant image of the specimen against an entirely dark background.

Limitations.—For the reasons mentioned above, the numerical aperture of the illuminator must be greater than that of the object glass.

NA of Object Glass.—When using an ordinary dry substage illuminator with an opaque stop, its maximum aperture will be unity, and this method can only be used if the numerical aperture of the object glass does not exceed 0·70 or 0·80 if the illuminator is fully corrected.

If the illuminator is oiled to the slide, the numerical aperture is correspondingly increased and the method can be extended to object glasses of slightly greater aperture.

It is then preferable to use specially designed oil-immersion dark-field illuminators, some of which can cut off a cone of $NA = 1{\cdot}30$.

Total Reflection.—When using these illuminators the light rays are so oblique that they will be totally reflected within the illuminator and will not emerge at all unless the top lens is oiled to the under surface of the slide. Similarly they will be totally reflected within the glass slide before reaching the specimen unless the latter is mounted in some fluid or medium of a refractive index near to that of glass.

Specimen must not be mounted dry.

Dry mounted specimens cannot therefore be examined with these special illuminators.

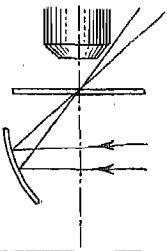
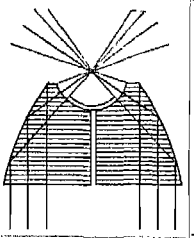
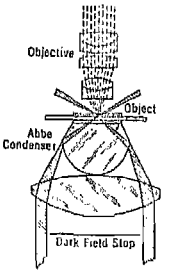
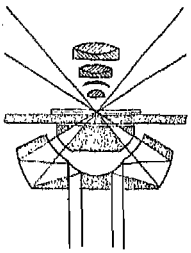
Once the oblique rays have penetrated to the specimen, however, their further course is unimportant (so long as they do not enter the object glass). What now matters for the formation of the image is the light scattered or reflected by the specimen itself. This explains why, although the dark-field illuminator must be oiled to the slide, it can nevertheless be used equally well with dry and oil-immersion object glasses.

METHODS FOR DARK-FIELD ILLUMINATION

The various methods are tabulated, together with their characteristics, in Schedule II on page 87.

For the Lowest Powers (I).—An oblique pencil of light can be reflected from the surface of the concave mirror upon the specimen object glass lens. The mirror should be swung out of the optical axis and supplied with a beam of parallel rays. The method is very limited in its application.

Schedule II. DARK-FIELD ILLUMINATION

System.	Diagram.	Uses.	Advantages.	Disadvantages.
Oblique rays from concave mirror. See page 86.		Lowest powers.	Simplicity. Adjustable light angle. Easy to pass from transmitted to dark-field illumination, and vice versa.	Field is not truly dark, unless a dark well or black stop is arranged some distance below stage.
Spot lens (not shown) or Wenham type paraboloid. See page 88.		Low and medium powers.	Easy to adjust. Gives excellent results with a variety of objects.	Field is never really dark. Unsuitable for use with objectives of high power and short focal length.
Standard substage illuminator with stops. See page 88.		Powers up to 4mm, but *NA* must be less than 0·7 for Abbe, or 0·85 for fully corrected illuminator.	Simplicity. No special apparatus. Easy to change over to transmitted illumination. Can be used with coloured stops for Reinberg effects. Can be used with dry mounts.	Some light gets past the stop, because the stop is some distance below the illuminator, hence field is never really dark. Not suitable for high powers. *NA* of objective must not exceed 0·70–0·85.
High power dark-field oil-immersion illuminator. See page 89.		Highest powers. Especially for small living organisms, colloidal particles, etc.	Only satisfactory system for high powers. Very dark field. Very brilliant illumination.	Dry mounts cannot be used. Slide must be thin. Adjustments are delicate. Generally objective *NA* must not exceed 1. Modern illuminators can cut off a cone of *NA* = 1·30 but the mounting fluid must then have an *RI* > 1·45 which precludes examination of living specimens.

Wenham Type Paraboloid (II).—The paraboloid, though old-fashioned, gives very good results with any power not exceeding $\frac{1}{3}$ inch. It is easily adjusted, free from diffraction effects, and very generally useful. It is difficult to obtain a paraboloid of this type to fit the modern substage, and this is a pity. The neglect into which this device has fallen is unjustifiable and regrettable. The principle will be readily understood from the illustration in Schedule *II*.

Standard Substage Illuminator with Opaque Stop (III).—The most generally useful method is to use the standard substage illuminator with an opaque stop of suitable size inserted in the stop carrier just below (or sometimes above) the illuminator iris diaphragm (see Fig. 24).

Precautions.—Certain precautions must be observed:

(*a*) As in all dark-field methods, the cover slip and slide must be absolutely free from dust. Any small specks will scatter light and confuse the image.
(*b*) The aperture of the illuminator must be greater than that of the object glass.
(*c*) The illuminator may be oiled to the slide in order to increase the numerical aperture but in this case the specimen must be mounted in a medium of suitable refractive index.
(*d*) The beam of light supplied to the illuminator should be approximately parallel.
(*e*) The illuminator must be very accurately centred.

Instructions for Adjustment.—A simple method of adjustment is first to obtain critical illumination in the usual way. An opaque stop can now be selected by trial of such a size that, when looking down the tube with the eyepiece removed, the light is just (but entirely) cut off. Replace the eyepiece and open the illuminator diaphragm fully. Readjust the position of the illuminator very carefully until the specimen shines out brightly against a dark field. If the ground is not quite dark, try a slightly larger stop. The higher the power and numerical aperture of the object glass, the more delicate the adjustment. Some light will always get past the stop, because it is at some distance from the illuminator lens, so that the field will never be truly dark.

It is a simple matter to revert immediately to transmitted illumination on removing the stop and readjusting the diaphragm. This is a very valuable feature.

Rheinberg Effect, or Differential Illumination.—The standard stop used gives a black central disc surrounded by an annulus of white light. If we substitute a stop consisting of a transparent central disc of dark blue (for instance) surrounded by a transparent annulus of light red, the specimen will appear coloured bright red against a dark blue field. Any combination of colours may be used, but the central disc must be dark and the annulus relatively light.

If the annulus is divided into four equal segments, opposite segments being of the same, and adjacent segments of different colour while a third colour is selected for the central disc, remarkable contrasts can be obtained. If the stop is suitably oriented, transverse structure will be illuminated in one colour and longitudinal structure in another, the whole against a background of a third colour. The method is useful for increasing contrast, and for photomicrography.

Special Dark-Field Illuminators Oil-Immersion only (IV).—This (like the paraboloid) is a *reflecting*, as distinct from a refracting, device by means of which a very small image of the source of illumination is focused upon the object, and this image is formed by rays of light which fall upon the object at an angle sufficiently oblique to be outside the aperture of the object glass. The *whole* of this light is so oblique that it will not emerge at all, owing to total reflection, unless the illuminator is oiled to the slide. Any dry or immersion object glass may be used so long as the numerical aperture *does not exceed unity*. (With specially designed illuminators, apertures of 1·20 or even 1·30 are permissible when using a 0·5-mm. glass slide.)

As already stated, the specimen must be mounted in a medium of refractive index similar to that of glass (the lower limit of the refractive index is 1·45 for a cone of 1·20 aperture).

The object must lie *exactly* at the crossing-point of the beams of light—or it will not illuminate at all, whereas when using an ordinary substage illuminator and stop, the object will still be illuminated if not in the exact focus, but not so brilliantly.

Focusing Troubles.—Since the illuminator is oiled to the slide and has a very short focal length, there is very little scope for adjusting the focus, and the thickness of the glass slide is an important consideration. The only possible remedy is to use an illuminator with a self-contained focusing adjustment which

increases or reduces its focal length according to the thickness of the slide (as illustrated in Schedule II).

Instructions for Adjustment.—The first essential is a very bright source of light of very small area. The "Pointolite" lamp is ideal for this purpose.

The glass slide is oiled to the upper surface of the illuminator whatever the power of the object glass and whether it is of the dry or of the immersion type. First place a $\frac{2}{3}$-inch object glass in the nosepiece and focus the specimen. Usually a ring of light will be seen in the field, and this may be appreciably out of centre. Centre this ring accurately by means of the substage adjustment screws. The appearance of a ring indicates that the intersection of the rays does not coincide with the plane of the object. The focusing adjustment of the illuminator is now adjusted until the ring contracts to a point.

If the illuminator is of the non-focusing type, this adjustment may be effected by careful raising or lowering the substage mount, but, on account of the small latitude, this is not always possible if the glass slide is unusually thick or thin.

The proper object glass is now substituted and focused. A very slight readjustment may be necessary (particularly centring) and it may be an advantage to reduce the aperture of the object glass slightly, in order to darken the field. This is done by means of an iris diaphragm carried in an auxiliary fitting interposed between the nosepiece and the object glass. This is known as a Davis diaphragm and is very valuable for use with all forms of dark-field illumination on account of the importance of the relationship between the apertures of the object glass and dark-field illuminator.

Use of Davis diaphragm.

REFLECTED ILLUMINATION

for opaque objects

Working Distance the Decisive Factor.—When opaque objects are to be examined, the substage mirror or illuminator can no longer be used. The illumination must be derived from rays of light thrown on to the specimen from above, and thence reflected into the instrument. Here, the governing factor is usually the working distance of the lens.

Condensing Lens.—With low powers, and correspondingly large working distances, the arrangement shown first on Schedule III

is satisfactory. If the working distance is too small for convenience, an interesting variation of this method can be used. This is shown next in Schedule III. The condensing lens is used partly as a total reflection device so that an extremely narrow beam of great intensity can be concentrated on the specimen. The condensing lens must of course be of the plano-convex type.

A parabolic reflector can also be used, as shown in Fig. 16 but this is an awkward arrangement since the reflector gets in the way of the stage and focusing adjustments.

Lieberkühn.—In all cases so far mentioned, the illumination is unilateral. Annular illumination can be obtained by means of a paraboloid reflector known as a Lieberkühn arranged around the nose of the object glass. An incident parallel beam is reflected upwards from the plane side of the substage mirror, and reflected back upon the object. The specimen must be mounted on an opaque patch in the centre of a glass slide. With this type of illuminator an object glass of even shorter working distance can be used, but a separate reflector, of appropriate focus, is required for each object glass. This is a serious drawback.

Vertical Illuminators.—When using high powers with very short working distances it is necessary to use some type of vertical illuminator (Figs. 25, 26, and 27).

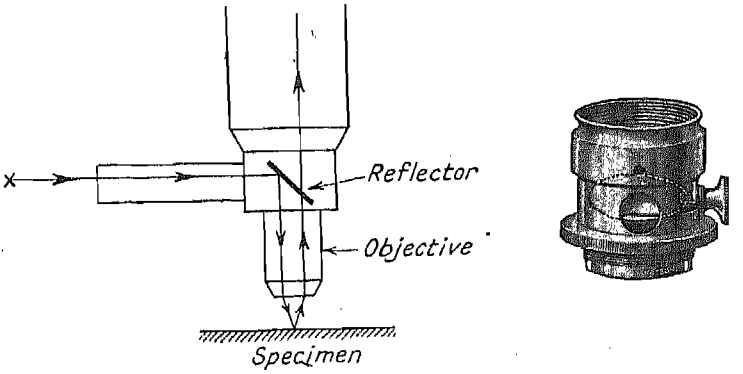


Fig. 25.—Vertical illuminator, mirror type (Watson).

The vertical illuminator consists, in its simplest form, of a plain optical glass reflector (usually a cover slip) held at an angle of 45° in an auxiliary mount immediately behind the object glass. A beam of light is thrown upon this reflector through an opening

in the side of the fitting and is thus reflected down through the object glass upon the object, from which it is reflected back into the object glass.

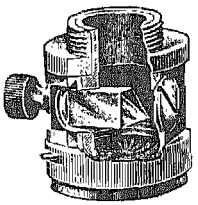


FIG. 26.
Vertical illuminator, prism type (Watson).

The object glass thus acts as its own illuminator, and, being focused in the usual way, a brightly illuminated field will result.

Sometimes the plain glass reflector is replaced by a small prism which is arranged to project over part of the aperture of the lens (Fig. 26).

The mirror type gives less light, but does not interfere with the lens aperture.

The prism type gives more intense illumination, but seriously affects the performance of the lens.

The distance from light source to illuminator should be of the same order as the distance from the reflecting surface of the illuminator to the eyepiece diaphragm.

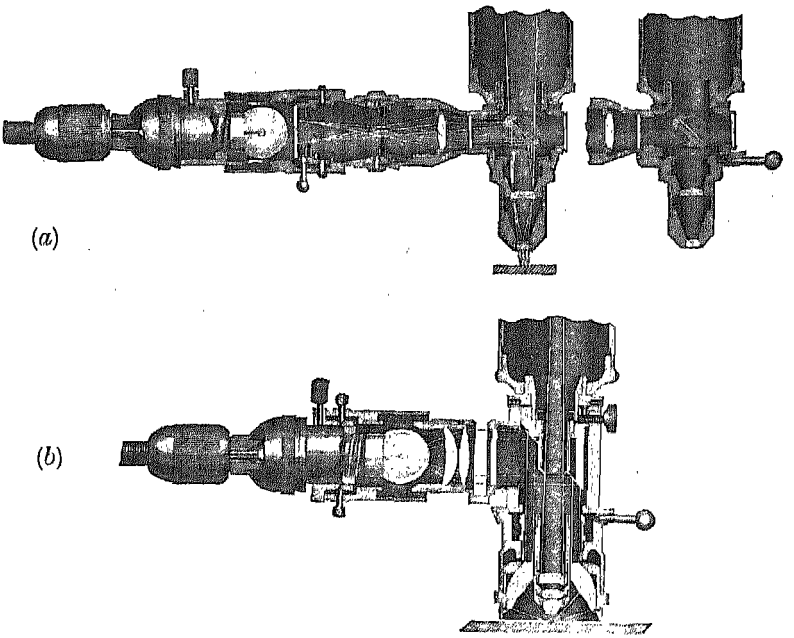
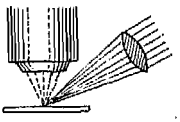
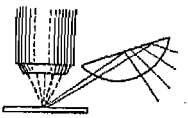
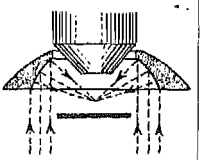
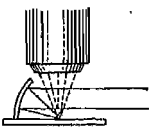
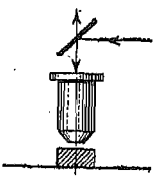
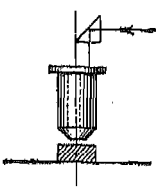


FIG. 27.—Two types of vertical illuminators of the combined unit type. (Cooke, Troughton & Simms, Ltd.)

(*a*) Normal incident illuminators, substituted for the objective changer.
(*b*) Oblique incident illuminator requiring specially constructed objectives.

Schedule III. REFLECTED ILLUMINATION

System.	Diagram.	Uses.	Advantages.	Disadvantages.
Condensing lens. See page 90.		Low powers.	Brilliant illumination.	Only possible with an objective of considerable working distance. Side illumination only.
Condensing lens adjusted for a narrow beam. See page 91.		Medium powers.	Owing to the very narrow beam, objectives of shorter working distance can be used.	Correct adjustment is rather delicate. Side illumination only.
Lieberkühn. See page 91.		Up to medium powers.	True symetrical overhead illumination.	Special reflecting paraboloid required for each objective.
Side reflector. See page 91.		Up to medium.	Intermediate between first and second systems.	Awkward to handle and adjust unless the working distance is considerable.
Vertical Illuminators. (*a*) Mirror type.		General purposes. Metallurgy.	True vertical illumination. Does not reduce *NA* of objective.	Causes some glare. Illumination rather faint.
(*b*) Prism type.		As above.	Reflects more light.	Reduces *NA* of objective.
(*c*) Annular type. See pages 91 and 94.	See Fig. 27 (*b*).	General purposes. High powers.	Carry own light source. No glare. Intense light.	May require special objectives to fit inside annular lens.

An iris diaphragm, in front of the lamp, controls the size of the illuminated area on the specimen, thus reducing the glare which is one of the disadvantages of these devices.

By using a suitable condensing lens, the lamp may be brought up close to the illuminator. This is utilised in some illuminators which carry, in a metal tube at right angles to the microscope axis, the condensing lens, iris diaphragm, and a small incandescent bulb, as a complete illuminating unit. The advantage of this arrangement is that the lamp adjustments are not disturbed when the instrument is re-focused. On the other hand, unless it is very light and compact, it undoubtedly throws undue strain on the nosepiece (Fig. 27).

Annular Type.—Of late years, illuminators have been developed which not only carry their own light-source but reflect an intense spot downwards through an annular lens surrounding the object glass. This greatly reduces glare.

The various forms of reflected illumination are summarised and compared in Schedule III on page 93.

OBLIQUE LIGHT

The reader who studies the more important text-books on microscopy written towards the end of the last, or the early years of this century will be struck by the importance attached to oblique light and the many strange devices designed for this purpose. Some of them were very ingenious, but none has survived.

Oblique light nevertheless has its uses, but it would in any case be proper to include it here if only to save the reader of these text-books a great deal of perplexity. They contain a great wealth of valuable material, and much also which is misleading and obsolete.

First of all, let it be clearly understood that:

(1) A wide, strictly axial cone of rays is *the only reliable method* of direct or transmitted illumination, and the results obtained by any departure from this method are to be regarded with suspicion.

(2) Oblique light here means the use of a narrow cone or beam of rays directed upon the object from any direction other than the optical axis *provided that it enters the object glass.*

(3) Since a properly computed substage illuminator is of

sufficient aperture to fill an object glass of equally high aperture, it is obvious that it can produce the most oblique rays which satisfy the above definition of oblique light. All special devices such as tilting stages, swinging illuminators, etc., are thus entirely useless.

(4) Closing down the substage diaphragm to a very small aperture *after* it has been swung out of centre for oblique light, or using the equivalent arrangement of a stop with small apertures or notches on the periphery does not reduce the useful aperture of either object glass or illuminator. Both are being utilised to the fullest possible extent.

Diffraction Theory of Microscopic Vision.—When an object such as a diatom, which has a large number of symmetrically disposed and evenly spaced markings, is examined by transmitted light, it will be seen, on removing the eyepiece and looking at the back lens of the object glass, that the light consists of a central beam of white light surrounded by a certain number of symmetrically disposed spectra. These are visible towards the outer edge of the lens, and are blue towards the optical axis and red towards the outer periphery of the lens. They are most easily seen with a specimen of *Pleurosigma angulatum* focused on the stage.

The number and arrangement of the spectra depend on the pattern of the markings, and their distance from the central beam is greater the finer the markings on the specimen.

According to the diffraction theory of microscopic vision, a true image will result only if all the spectra are collected by the object glass and thus re-combined with the central beam in the final image. If an annular opaque stop is placed over the back lens so as to cut off the spectra while leaving the central or dioptrically produced beam unaffected, all the markings are seen to vanish and the specimen appears as a structureless outline.

If one only of the spectra is included with the central beam, the markings partly reappear and their nature (and even the number per unit of length) alters according to the number of spectra admitted or cut off.

The spectra are formed by rays of light diffracted at an increasingly oblique angle, and since their distances from the optical axis increase as the markings get finer, it follows that lenses of increasingly large aperture will be required to collect them and thus resolve the finer markings.

When dealing with very fine periodic markings such as the "dots" on the diatom *Amphipleura pellucida*, which may number 95,000 to 100,000 to the inch, the spectra are so far out that the highest existing aperture is insufficient to include any of them

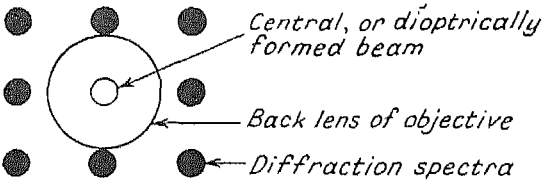


FIG. 28.

under normal lighting conditions, so that the dots are unresolved and invisible. Fig. 28 shows the central beam, spectra, and field covered by the back lens of the object glass under normal conditions of illumination.

By inserting an opaque stop under the illuminator provided with a small aperture or notch on the periphery at "six o'clock", the object will be illuminated by a very oblique beam sloping upwards. The effect is to shift the central beam across to the top

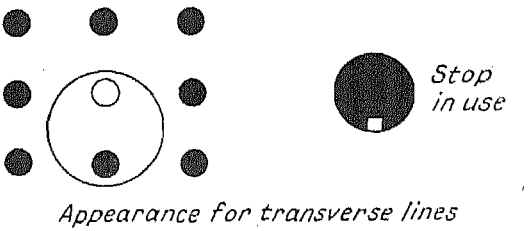


FIG. 29.

of the field and with it admitting one of the spectra at the lower edge, as shown in Fig. 29, since the distance from central beam to spectrum is constant.

When examining *Amphipleura pellucida* with its long axis arranged up and down the field of view, it will be seen that a series of transverse horizontal striae are now revealed.

With the arrangement shown in Fig. 30, on the other hand, these will be replaced by an exactly similar series of longitudinal or vertical striæ.

If we use a stop combining both notches, as shown in Fig. 31, we shall include a portion of two spectra, one on the horizontal and one on the vertical axis of symmetry. It will now be seen that the diatom is resolved into a series of minute dots lying at

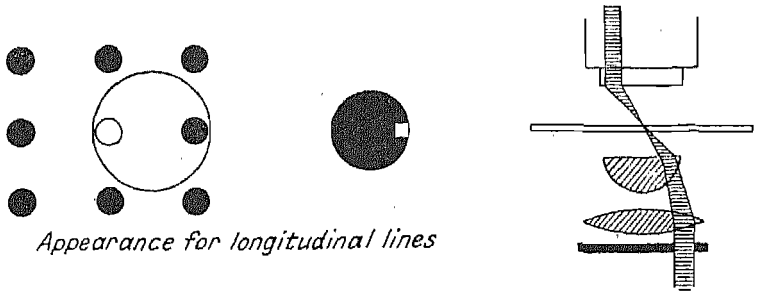


FIG. 30.

perfectly regular intervals along the transverse and longitudinal lines previously observed as independent series of striæ.

It is of course doubtful whether the structure so revealed bears any relationship to reality, or whether, according to some authorities, such markings exist at all!

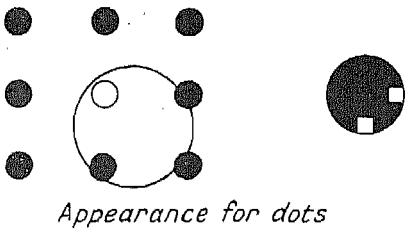


FIG. 31.

Nevertheless very fine structures of known nature such as Nobert's lines which run to 125,000 to the inch cannot be resolved by any other method.

In any case the resolution of *Amphipleura pellucida* into dots is an excellent test for both microscopist and instrument. Unless every adjustment has been correctly made, resolution is impossible. It is also one of the severest tests for photomicrography.

CHAPTER VII

THE MICROSCOPE IN USE

SUCCESSFUL results with the microscope demand comfortable working conditions, concentration, and unhurried attention to systematic adjustments.

Whatever problem is being investigated must be clearly defined beforehand so that the observer knows just what he is required to do; knowing this he can decide what apparatus he will need, and on this again will depend the nature and degree of precision of the adjustments he will have to make.

It is wasteful to use an elaborate set-up for the routine examination of coarse specimens, but it is just as inefficient to undertake high-power work for the purpose of revealing fine structure with makeshift arrangements in the hope that it will be "good enough".

According to some authors no satisfactory results can be expected unless the most elaborate procedure is rigorously applied. Other authors will tell you to screw an object glass into the nosepiece, drop a simple illuminator into the substage fitting, pull up your reading lamp into a convenient position, and get on with the work.

Both are probably right, and both are undoubtedly experts, but they are not talking about the same thing. The microscope is an extremely adaptable instrument with several degrees of efficiency and considerable latitude is permissible. Such latitude may be dangerous for the student, without due warning, since he will only be in a position to simplify procedure effectively when he has learned to appreciate the difference between a critical image and one that is not.

It is essential to know how to obtain the maximum out of the instrument and every one of its components before one can decide where and how to simplify. It is a mistaken policy to use rough and ready methods at the outset for want of knowledge and practice; it is too easy to fall into slipshod habits of work.

It is proposed to deal in turn with general conditions, illumination, optical components, corrections, tests, faults, and maintenance.

GENERAL CONDITIONS

The first essential is a comfortable seat with a spacious table of suitable height. Plenty of room is a great convenience, and cramped conditions are a serious handicap. It is a great mistake to have accessories and optical components littered about the table. Such conditions lead to irritation and impatience which are fatal. Decide beforehand approximately what apparatus you will require and put the remainder away.

By far the best arrangement is to procure a fairly large dust-proof metal box (the type used for surgical dressings is ideal) which should be at least 4 inches deep. A piece of $\frac{1}{4}$-inch wood is now cut to fit the box and arranged to rest on supports so that it forms a flat surface or tray raised about $2\frac{1}{2}$ inches from the bottom. Borrow an adjustable bit and brace and cut circular holes through the wood to take all optical components. The eye-pieces will slip in resting on their flanges and the objective boxes will rest on the lid flanges. Larger holes can be provided for substage illuminators, but the more highly corrected types are usually contained in metal boxes similar to those supplied with objectives. It is an advantage to allow plenty of room so that new components can be added progressively. The components can be arranged in order of magnification, with a row for the achromats and another for the apochromats, etc. The result is very neat and extremely convenient. The box takes up little room on the table, by the side of the instrument, or if the table is small it can be placed on a ledge below the table. Any component can be picked out as required and easily dropped back into position after use.

If desired, a row of compartments can be provided along one side of the box for such accessories as filters, graticules, etc. (Fig. 32).

The room should be partly darkened or, if this is impossible, suitable screening should be provided. Unscreened windows and other light sources are very distracting and may give rise to unwanted reflections from the substage mirror which sometimes cause trouble and interference.

The ideal is to have two lamps, a simple one of the frosted bulb type and a high intensity lamp fitted with a condensing lens. Both should have filter carriers and iris diaphragms. We will return to this point later.

It is an advantage to have the table up against a wall. A glass-fronted cabinet can be fitted to the wall over the table in which extra apparatus can be kept, and even the microscope itself.

It is a good plan to have a small board fitted to the wall with a number of electric sockets and a switch. Various types of lamps or illuminating apparatus can be plugged into these sockets as

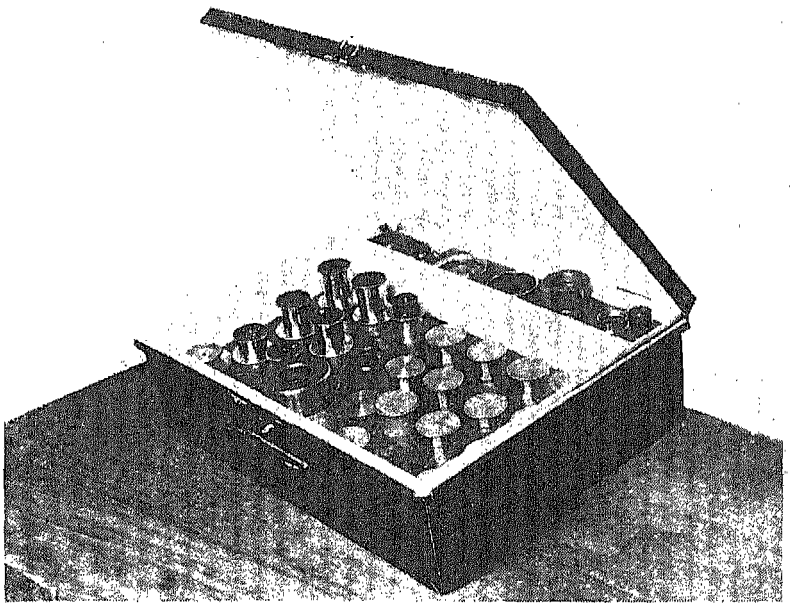


FIG. 32.

required. Half the sockets should be connected to the 220 V mains, and the other half to the secondary of a 220/6 V, 5-ampere transformer. These 6 V sockets should be *smaller* than the standard sockets (i.e. the plug pins should be closer together) so that 6 V illuminating devices cannot be plugged into the 220 V points by mistake.

All mounting activities and chemicals generally should be excluded, a separate table and cupboard being used for this purpose.

Dust is the worst enemy, and provision should be made for all apparatus to be put away or adequately covered by means of glass bell-jars, dust sheets, etc.

THE INSTRUMENTS

If at all possible it is a great convenience to have two microscopes: a simple stand with a simple substage illuminator of rather low aperture, and 16-mm. and 24-mm. objectives mounted in a double nosepiece for preliminary work, and a really elaborate stand, as complete as requirements will justify, for the actual work itself.

Instruments are usually so adjusted that when the objectives are mounted in the revolving nosepiece, the optical axis of each is co-axial with that of the microscope as a whole, and also that they are as nearly parfocal as possible, that is to say, on changing from one objective to another, the re-focusing required is reduced to a minimum.

A word of warning is necessary here. Each objective (assuming, of course, that they have been supplied with the stand) must be inserted into the opening for which it has been centred. The openings and objectives bear corresponding markings. Since, in some instruments, the fine focusing adjustment affects the position of the objective only, and does not move the main body, it is impossible for the objectives to be precisely parfocal. In such cases the objectives are arranged to be parfocal when the fine focusing adjustment is at the middle of its range. Finally, great care should be taken when passing from a low to a higher power lens which will have a much shorter working distance. The front lens of the objective or part of the mount may catch on part of the mechanical stage, or the spring clips, or even on the cement ringing the cover slip. These obstacles may be easily cleared by one lens but may damage the next.

If the instrument is used habitually with objectives supplied by different makers, the revolving nosepiece is inconvenient and of no advantage. In such cases it is better to dispense with it altogether and re-centre the substage where necessary or, alternatively, to use the sliding type of changer by means of which each objective can be accurately centred once and for all. Sets of these changers are unfortunately expensive. There exists a type of revolving nosepiece in which each objective can be centred by means of adjustment screws, but it is rather heavy.

Some instruments are fitted with an inclined eyepiece, so that the stage is always horizontal. It is not then practicable to

contain the effective tube length within 160 mm., and the system is extended to some such value as 210 mm., the additional tube-length being compensated for by the introduction of an extra lens within the eyepiece tube. It may be necessary in such cases to multiply the overall magnification obtained from the values indicated on eyepiece and object glass by a factor (1·5 in this case) given by the manufacturer.

The inclined eyepiece, whether monocular or binocular, is an advantage for certain types of work when the stage must be kept horizontal. This applies particularly to hanging drop methods and to examinations in liquids generally. In such cases the ordinary vertical tube is inconvenient and very tiring.

Inclined eyepieces may be of fixed inclination, and in such cases the height of the seat in relation to the table is critical. An adjustable stool (such as a piano stool or some models of typist's chairs) is very helpful. Other inclined eyepieces can be varied from 0 to 45°, and this type, though more expensive, is far preferable.

The modern tendency is to equip all research microscopes with inclined eyepieces.

The setting-up of the instrument itself presents no special difficulties. If the instrument is new, and is being used for the first time, the manufacturer will have supplied an instruction booklet, and this should be carefully studied beforehand. It is important to become entirely familiar with all adjustments and their positions so that they can be handled without fumbling or hesitation while looking through the instrument.

Both eyes should be kept open. It is the eye not in use which becomes tired. An eyeshade arranged to clip on to the bodytube is very helpful and can easily be improvised, though most makers supply them.

Remember that any additional device such as an objective changer, nosepiece diaphragm, vertical illuminator, etc., will increase the mechanical tubelength and will require compensation by means of the drawtube.

ILLUMINATION

This has been fully dealt with in an earlier chapter.

For convenience, and concentrating on one particular method of transmitted illumination generally applicable to most classes of work, the main points can now be summarised as follows:

(I). **Lamp.**—There are two types:

(1) An extended uniformly illuminated surface such as an opal bulb or frosted glass for low or even medium powers, and routine examinations.

(2) A high intensity compact light source used in conjunction with a lamp condensing lens, for high powers and more exacting work.

(II). **Substage Illuminator.**—There are three types:

(1) The aplanatic (or even the two-lens Abbe) for preliminary or general routine work.

(2) The achromatic, for more exacting work and preferably for all semi-apochromats and apochromats.

(3) The specially corrected wide aperture type for oil-immersion.

It always pays to use an achromatic condenser, and it is not much more expensive than the aplanatic type. The *NA* of the condenser must be at least equal to that of the objective with which it is used.

(III). **Conditions.**—The ideal conditions are:

(1) That a sufficient area of the object should be illuminated, and no more. Both types of lamps should therefore be fitted with iris diaphragms.

(2) That an image of the light-source, or more exactly of the frosted surface in the first case and of the lamp condenser iris in the second, should be focused in the object plane by means of the substage illuminator, so that the illuminated object behaves as if it were self-luminous.

(3) That the back lens of the objective should be evenly filled with light. This condition is controlled by adjusting the substage diaphragm.

(IV) **General Set-up.**—Normally the purpose of the condensing lens is to increase the apparent area of the source of light *and not its intrinsic brilliancy.*

It is also used for paralleling the rays, or for focusing an image of the light-source on the substage iris for Köhler illumination.

It can exceptionally be used to concentrate an intense spot of light on some portion of the specimen.

It is a great advantage to have an aplanatic condensing lens.

The opal bulb or frosted glass type of lamp has no axis in the optical sense so that its exact position in relation to the instrument is unimportant.

When the high intensity lamp and condensing lens are used, on the other hand, there is a true optical axis and careful adjustment is required.

The best method for general work, and the easiest to set up and adjust is the Köhler method.

The lamp and lamp condensing lens will be correctly adjusted in relation to the instrument when an image of the light-source (*not* the condensing lens iris) is formed on the substage iris, the distance between the latter and the lamp condenser being approximately 10 inches.

The image *of the lamp condenser iris* is now focused in the object plane and the field of view will appear evenly illuminated. The area so illuminated is controlled by the lamp condenser iris, which should be adjusted so that its image just disappears from the field of view. The *substage* iris is used to ensure that the back lens of the objective is just full of light.

This is the most convenient and widely used arrangement for transmitted light. Modifications and refinements of this method may be required for exacting work, and details will be found in the chapter dealing with illumination.

The *intensity* of the light may be adjusted by interposing one or more neutral filters in the filter carrier or by means of a rheostat.

OPTICAL COMPONENTS

The illumination problem being satisfactorily solved, the only remaining difficulty is the choice of a suitable object glass.

Once this choice has been made, the type of substage illuminator will follow, depending on the quality of the object glass and its numerical aperture.

The first rule is to use the lowest magnification which will reveal the detail required to the eye in such a way as to be comfortably visible.

Thus the main consideration will be numerical aperture. If the interval to be resolved is of the order of 0·5 microns, for instance, we know that a lens of 0·5 *NA* will resolve this (see Table, p. 37). We would allow a fair margin, however, and probably use a 0·65 *NA* lens, which is the standard value for a 4-mm. achromat or an 8-mm. apochromat with initial magnifications of 40x and 20x respectively.

With a 10x eyepiece, this would represent overall magnifications of 400x and 200x.

The original 0·5μ would thus be magnified to 200μ or 100μ, but 100μ is at the limit of what the eye can perceive so that the 4-mm. lens would be required as a minimum for this eyepiece.

The correct choice thus depends, not on the *NA* alone, but on the corresponding magnification of the particular objective considered, and its ability to stand high eyepiecing without appreciable image deterioration.

We have also seen that a reasonable overall magnification for general work is 1000x the numerical aperture.

A study of the accompanying chart will make these points clear and explain how the various factors can be taken into account simultaneously.

Chart: *Magnification—Resolution—Numerical Aperture.*

The following chart is a summary, in practical form, of the main points raised in the chapter on numerical aperture.

(1) We have shown that the smallest interval between two points that can be resolved under normal conditions is of the order of 0·2 microns.

(2) In order that the human eye should be able to perceive this, a magnification of 500 diameters is required to bring it up to 100 microns.

(3) The numerical aperture required to resolve 0·2 microns is

$$NA = \frac{0{\cdot}5\lambda}{R} = \frac{0{\cdot}5 \times 0{\cdot}55}{0{\cdot}2} = 1{\cdot}37.$$

(4) The *minimum* magnification required is thus approximately equal to 360 times the numerical aperture (500/1·37 =360).

(5) For comfortable vision, a factor of 3x should be allowed for, so that, as a general rule the useful maximum magnification for comfortable vision can be of the order of 1000x the value of the numerical aperture.

Numerical apertures are given on the scale at the top of the chart, and the corresponding overall magnifications based on 1000x *NA* are given on the lowest scale. A third scale gives corresponding values for the interval resolved.

Six object glasses have been chosen as typical of their class, and the ranges of magnification are plotted against them, together with the corresponding eyepiece magnifications.

The vertical arrows give typical values of the numerical aperture for such lenses; the dotted lines correspond to achromatic lenses of high quality, and the plain lines to apochromatic lenses of the same power.

It will be seen that high quality achromatic lenses and especially apochromatic lenses will stand considerable eyepiecing, or further magnification of the image in any form (as on a photographic screen), even if the 1000x *NA* rule is observed. As a matter of fact, apochromatic lenses will stand overall magnifications of 1500x or even 2000x the value of the numerical aperture, though this is only useful for photographic work.

Use of Chart.—It is first assumed that you have drawn up a chart of this nature incorporating all objectives and eyepieces at your disposal.

(1) Consider the minimum interval you wish to resolve. Let us suppose it to be of the order of 0·40μ. Mark off this value on the lower, or resolution scale.

(2) Read off the corresponding *NA* required on the upper scale, which will be 0·70.

(3) Follow the vertical line joining these two points till it intersects the first horizontal object glass range line. This will be a 4-mm. 0·85 *NA* lens.

(4) Find the correct eyepiece along this line, which will be the nearest to the right of the point of intersection, in this case 20x. The corresponding overall magnification, 800x, is obtained by following the vertical from the 20x point to the magnification scale.

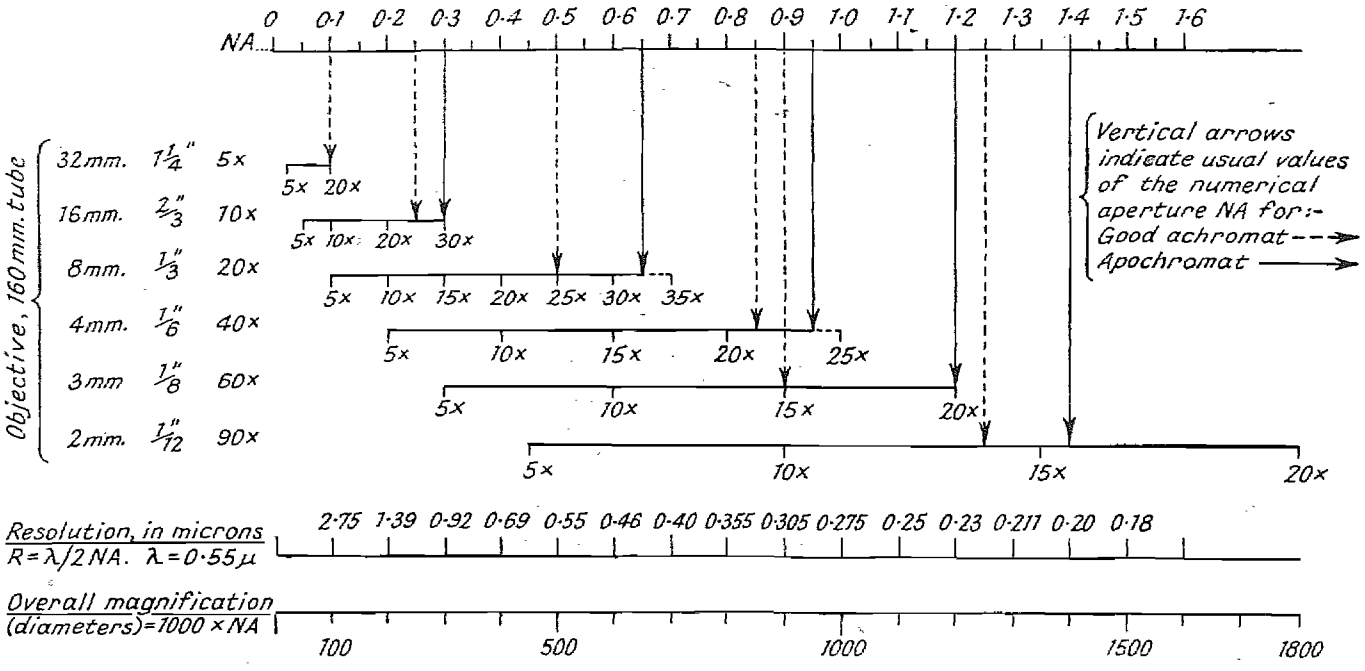
You may prefer to follow the 0·7 *NA*–0·4μ vertical line further to the next intersection, giving a 3-mm. 0·9 *NA* objective. The correct eyepiece will now be 15x and the magnification 900x.

As a third alternative you may decide to use a 2-mm. 1·25 *NA* lens giving the same overall magnification with a 10x eyepiece, but this will be an oil-immersion lens.

The choice between these alternatives will depend on such considerations as depth of focus and working distance (both of which will fall off very rapidly as the initial magnification of the objective increases), the size of the field of view, an important point if you want to observe some detail in relation to its immediate surroundings, delicacy of adjustments, necessity for oil-immersion, type and *NA* of illuminator in use, and general convenience.

In the example chosen, the most suitable combination would probably be the 3-mm. lens with a 10x eyepiece.

The question of working distance may be of importance, particularly in the case of overhead illumination or special applications involving focusing the inside of vessels, or dangerous


NA
0 0·1 0·2 0·3 0·4 0·5 0·6 0·7 0·8 0·9 1·0 1·1 1·2 1·3 1·4 1·5 1·6
Objective, 160mm. tube
32mm. 1¼" 5×
16mm. ⅔" 10×
8mm. ⅓" 20×
4mm. 1/6" 40×
3mm 1/8" 60×
2mm. 1/12" 90×
5× 20×
5× 10× 20× 30×
5× 10× 15× 20× 25× 30× 35×
5× 10× 15× 20× 25×
5× 10× 15× 20×
5× 10× 15× 20×
Vertical arrows indicate usual values of the numerical aperture NA for:-
Good achromat ---→
Apochromat ——→
Resolution, in microns
R=λ/2NA. λ=0·55μ
2·75 1·39 0·92 0·69 0·55 0·46 0·40 0·355 0·305 0·275 0·25 0·23 0·211 0·20 0·18
Overall magnification
(diameters)=1000×NA
100 500 1000 1500 1800

proximity to objects giving off heat or corrosive fumes. It may prove a limiting factor when examining specimens mounted in liquid, if the cells are deep or, in the case of high powers, if the cover glass is unusually thick.

The field of view depends on the magnifying power of the objective, and on the diameter of the diaphragm in the eyepiece at the plane of the real image. It becomes very small for high magnifications, and the revolving nosepiece is here used to the best advantage. It enables the skilled observer to pass rapidly and easily from one objective to another thus getting a sort of composite picture of the specimen as a whole, together with its structural detail, and a correct appreciation of their relationship.

Much the same considerations apply to the depth of focus, or penetration, as it is sometimes called, but in a different plane. It has been said that the depth of focus of a high-power lens is really the fine focusing adjustment. This adjustment, in the hands of a skilled observer, is in constant motion focusing first one plane, then another. He thus again builds up a composite picture from a number of thin optical sections, thus gaining a perception of depth which cannot be otherwise obtained.

The depth of focus, which is inversely proportional to the numerical aperture and the magnification, can be increased only by correspondingly decreasing either one or the other.

When taking photomicrographs, the depth of focus is very much reduced and the photograph will only represent a very thin section of the specimen.

Unless the specimen is itself an extremely thin object, much of it will be out of focus to varying degrees, and may interfere with the general quality and sharpness of the picture. In this case depth of focus is a very important factor.

For visual observation the true depth of focus, which may be defined as the distance between two optical sections of the specimen which are simultaneously sharply defined, is appreciably increased by the power of accommodation of the eye, but this is not the case for photography. The photographic depth of focus is only about a quarter of the visual depth.

CORRECTIONS

It has been explained that the use of a cover glass affects the definition of the image. Refraction of the cone of rays by the cover glass introduces a certain amount of spherical aberration,

so that a lens corrected for an uncovered object will be found to be over-corrected. To counteract this error it is necessary to introduce some spherical aberration into the lens or, in other words, to correct the spherical aberration for the lens and a cover glass of specified thickness considered as a combined optical unit. Modern objectives are corrected for a cover glass thickness of 0·17 or 0·18 mm., the exact value being almost always stated by the manufacturer.

It often happens that the cover glass is not of the specified thickness, or it may be necessary to examine an uncovered preparation. Some form of compensating adjustment must therefore be provided. The effect is unimportant for lenses of a focal length greater than 16 mm. or $\frac{2}{3}$ inch, but with a 4-mm. lens a variation of 0·01 mm. in the cover glass thickness will affect results. Apochromats, being more accurately and completely corrected, are even more sensitive. Oil-immersion lenses, however, are unaffected because the medium between specimen and lens has approximately the same refractive index throughout. Correction is obtained:

(1) By means of a correction collar altering the space between the back and front component lenses of the objective.

(2) By means of a lens corrector fitted between the nosepiece and the objective.

(3) By means of the drawtube, the effect of which is to alter the tubelength. Shortening the tube neutralises the over-correction introduced by a thick cover slide, and vice versa.

An objective is corrected for one particular tubelength only. It is important to understand the reasons for this. Any lens, or combination of lenses can be made to form an image of an object at many different positions. Any two positions where an object and its image are situated are called a pair of conjugate foci. The important characteristic of an object glass is that *it can only be absolutely corrected for one particular pair of conjugate foci*. Hence the importance of using the correct tubelength. Its importance has been greatly exaggerated by some authors, but for examining the finest detail with high-power lenses it is essential.

Since the objective is corrected for a specific value of the tubelength, and for a specific cover glass thickness, it follows that any variation in the latter can be compensated by altering the former.

Correction Procedure.—The clearest description of the procedure to be followed for lens correction is given in an article by J. Johnston, F.R.M.S., in the September 1931 issue of *Watson's Microscope Record*. I cannot do better than to quote the following extract:

"Select a lens of moderate aperture, say $\frac{1}{6}$ inch of 0·7 to 0·8 *NA* and use axial light. Place a slide on the stage; a diatom mount is very suitable, as there are generally particles of opaque foreign matter to be found dotted about here and there. (The idea is to practise the procedure on a convenient slide first, because, naturally, the corrections must finally be made on any slide under examination. It will be found that small specks can be found on any slide either as foreign matter, or as part of the finer structure of the specimen itself.) Pull out the drawtube about half-way, or, if you have a collared objective or a lens corrector, turn the collar to a midway position. Now focus the object and rack up the condenser and obtain critical lighting in the usual way. Now locate one of the indispensable black specks and bring it to the centre of the field. De-focus first upwards, then downwards and observe the effect. On one side of the focal point the dot will expand into a more or less well-defined ring, on the other side it will tend to vanish in a characteristic mistiness. Ignore the latter condition and concentrate on the ring formation. Again de-focus to the ring side and now correct, not by the fine adjustment, but by drawtube or collar. If the ring is formed on de-focusing downwards, pull out the drawtube or rotate the collar clockwise. If rings are formed on de-focusing upwards, shorten the tubelength or rotate the collar anti-clockwise. After several such adjustments it will be found that the rings become less and less well defined on one side and begin to appear on the opposite side of the focal point, until a stage is reached when rings, now comparatively faint, are equally apparent on either side of the focal point. This is the condition aimed at. In actual fact you will now get a beautifully clear and crisp image of the object."

To apply the test to dark-ground lighting is a simple matter once the test with transmitted light has been mastered. Lens adjustment is a lengthy process to describe, but with practice a few moments suffice and the movements are then carried out as mechanically as ordinary focusing.

TESTING OBJECTIVES

This is a matter for the expert. Various tests may be applied, sometimes a familiar biological specimen will be used, or a minute point as provided by a pinhole in a silver film will serve, but with such objects great experience and judgment are essential and the personal element is involved.

Nowadays, the interferometric test, using the Hilger Microscope Interferometer furnishes an impersonal method of great exactitude, and the pattern so obtained provides a valuable record of the residual errors of an objective. It is only mentioned here as a matter of interest.

The method produces interferogram bands, rather like the contour lines on a map, which indicate a difference of half a wavelength, or 0·00001 inch with green light of wavelength λ5461Å. An optical system may be considered perfect if the residual errors are below one-quarter of the wavelength of the light used, 0·000005 inch in this case.

Thus the interferogram obtained from such a system would be practically featureless. The examination is carried further to ascertain how the sub-tolerance errors are distributed. The central band of the interferogram gives substantially a graph of the actual errors of the lens as made and adjusted.

The diagram reproduced in Fig. 33 (British Scientific Instrument Research Association) shows such a graph obtained from a photographic interferogram of a 3·75 mm. fluorite (semi-apochromatic) lens of 0·95 *NA*.

Leaving these methods aside as belonging to the domain of the manufacturer and computer, there are nevertheless certain test objects which can give the experienced observer valuable information. This is particularly important when purchasing a second-hand objective, or where it is suspected that an object glass may have become damaged.

Unless the observer be in constant practice his eye may lead him astray. An object examined by day in a well-lighted room rarely appears the same as when looked at in the evening in a well-darkened room.

The secret of the successful use of test objects lies in complete familiarity with the particular slides used, and their frequent examination with different objectives under varying conditions of illumination. It would be futile, therefore, to attempt a

description, much less any sort of definition, of what the observer may expect to see. There are, however, a great many fine photo-micrographs of test objects as seen with the aid of different objectives, particularly in E. J. Spitta's *Microscopy*, Allen's *Microscope*, and Carpenter's standard text-book.

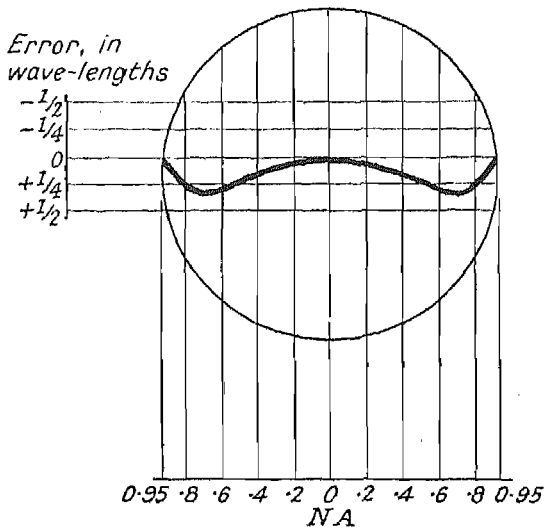


FIG. 33.

A study of these illustrations will give the reader some idea of what to expect.

A useful series of test slides is listed below:

(1) For low powers, up to and including 16 mm., the proboscis of the fly. This must be a good professional mount; if possible, one by Topping.

(2) For 8 mm., and particularly 4 mm., the pygidium of the flea. This is a good test even for a 0·95 *NA* apochromat.

(3) For the 4 mm., 3 mm., and 2 mm., diatoms are best. The following should be sufficient:

Surirella gemma, a very good test diatom.

Navicula lyra, useful for colour tests. It should show practically no trace of colour with a good apochromat.

Pleurosigma angulatum, a difficult diatom with a black and a white dot focus.

Amphipleura pellucida, with about 100,000 dots to the inch. A very difficult diatom to resolve without oblique green light, even with the finest objective.

Diatoms should be mounted in Hyrax, and *Amphipleura pellucida* in realgar if possible.

Test slides are useful for two reasons: as a test for object glasses of unknown quality, and as a test for the skill of the observer. In the early stages, when learning to set up an instrument and trying out different forms of illumination, test slides are convenient standards from which results may be assessed by comparison with photographs in more advanced text-books.

FAULTS

The modern microscope and its components are practically foolproof; once the fundamental principles have been mastered, successful work is a matter of practice. There are one or two common faults which frequently occur and may cause irritation and loss of time, but they are easily recognised and can be quickly put right. The following brief notes may prove useful in this respect.

First, remember that dust and dirt, greasy finger-marks, old oil and unclean slides are bound to cause trouble. Scrupulous cleanliness is essential to microscopy, as it is indeed to any form of scientific investigation.

Light.

Too strong.—Lower condenser (illuminator) slightly. Use neutral screens in filter carrier. Use variable resistance in lamp circuit.

Too faint.—Use condenser of higher aperture. Readjust position of lamp condensing lens.

Back lens will not fill with light.—The illuminating train may be out of alignment, particularly the mirror. The substage condenser may be of insufficient aperture. When using an oil-immersion objective, this may be due to the specimen being mounted dry.

Immersion Oil.

Immediately after oiling objective to slide, the field appears dark with moving shadows.—Bubble of air in oil.

Light suddenly becomes faint and fine structure vanishes.—The oil has left the space between the slide and the oil-immersion condenser.

Definition is poor,—Objective has been insufficiently cleaned and there is a thin film of hardened oil on the lens.

With a dry lens, as above.—The thin film of hardened oil is on the cover-slip.

Specks.—If specks appear in the field and float about, they are usually in your eye. If they are stationary, revolve the eyepiece; if the specks revolve with the eyepiece, clean the eyepiece lenses. If they do not move with the eyepiece they may be due to dust on the back lens of the objective which should be cleaned with a soft dry camel-hair brush.

If the image appears to move sideways when altering the focus, this may be due to improper alignment of the optical parts, but is more often caused by the plane of the specimen not being perpendicular to the optical axis. This may result from a faulty stage, or the slide may not be lying quite flat on the stage, or the specimen itself may be tilted within a rather thick layer of mountant.

Maintenance.—The importance of keeping the microscope in a perfectly clean condition cannot be over-emphasised. The working parts can be very sparsely lubricated with vaseline or clock oil.

Lens surfaces can be very easily damaged by the use of unsuitable cleaning materials. Well-washed cambric is best, or good quality lens paper.

Immersion oil should always be removed immediately after use. Use a very little Xylol for this purpose. On no account use alcohol or any other solvent. If the instrument has lacquered parts, alcohol will dissolve the lacquer. To clean such parts, a very little turpentine can be used, and the parts should then be wiped quite dry and rubbed over with a trace of vaseline, then wiped dry again.

Do not attempt to dismantle the instrument, or any part of it. Take it to a good instrument-maker or, better still, to the maker for repair or periodical overhaul. Never use force on any part of a microscope; if force is required, something must be seriously wrong.

Remember that bodytube and drawtube, particularly the latter, are easily dented or forced out of shape. Always leave an eyepiece in the tube to prevent dust from falling in on to the back lens of the objective. If you stand an objective on a table, place it upright, back lens down.

CHAPTER VIII

MEASUREMENTS AND COUNTS

ONE of the most common requirements in most forms of research or investigation is the measurement of microscopic objects. It often happens, as in the case of fungus spores, for instance, that the size is so constant for any given species as to constitute a valuable feature for exact determination.

Another important technique is counting. It may be required to determine the number of particles contained in a given volume of liquid, for instance, or the number of bacteria present, or, again, to detect the addition of maize starch to wheat flour, to estimate the purity of insect powders, the percentage of foreign matter in foodstuffs or drugs, etc.

Suitable modifications of these methods enable one to estimate the weight of such minute objects as pollen grains, fungus spores, grains of sand, etc.

Amongst the more important pathological applications are counts of red or white corpuscles in a blood sample, the examination of cerebro-spinal fluid or the enumeration of pus cells in urine.

MEASUREMENTS

The measurement of comparatively large objects presents no particular difficulties, but such is not the case with very small particles approaching the limits of resolution. They may be quite clearly visible, but it will often be found extremely difficult to decide just what has to be measured. These objects have no clearly defined edges, or at least there is always great uncertainty as to whether an apparently well-defined border is really the edge.

If the object is a slender filament, it may appear white with black edges. These may be due to diffraction. If the filament is very slender and the illuminating cone too small, there may appear a white line beyond the black one, and perhaps even a fainter black line beyond this again.

In the case of globular objects the appearances, when the focus is moved through different planes, depend greatly on the reflection and diffraction of light around the particle, and they by no means represent true plane or optical sections of the specimen. These

optical effects are very much in evidence when using oblique light or dark-field illumination.

There is no way out of this difficulty because it arises from the limitations imposed by the nature of light and the fundamental principles of microscopic vision.

There is one important rule, however, which will minimise, even if it cannot eliminate, these causes of error: *always use an objective of very high numerical aperture together with the widest possible illuminating cone.* If this should result in glare, or lack of definition and contrast, either of which may seriously interfere with the measurements, a considerable improvement can usually be obtained by interposing a suitably coloured filter between the light source and the instrument. On no account should the substage diaphragm be closed down beyond the point when it is just visible within the circumference of the back lens of the objective.

No such difficulty arises when we come to measure distances between small objects, even such minute distances as the interval between the "dots" on diatoms, for instance. Although we may not be able to decide what really constitutes the outer boundary of such a dot, we can always bisect it and determine its centre and hence measure the interval between this point and a corresponding point on the adjacent marking.

According to the general principles underlying microscopic technique, there are methods for rough approximation, and methods for extreme accuracy. It is inefficient and tiresome to use an elaborate method when a simple one will suffice.

Measurement by Means of the Mechanical Stage.—When the object is not too small (of the order of 200 to 100 microns) it can be measured with reasonable accuracy by means of the stage scales and vernier.

It is best so to arrange the object that the two principal dimensions, which will usually be at right angles to each other, lie along the directions of horizontal and vertical travel of the stage.

One edge of the object is now brought into contact with one edge of the field used as an index, and the vernier read. The object is now moved until the other end just touches the same or index edge of the field. The vernier is read again, and the difference between the two readings is the actual length of the object.

An improvement on this method consists in the use of a hair or index line in the focal place of the eyelens to which the two ends of the object are referred in turn, instead of the edge of the field.

The method is necessarily limited to fairly large objects because the average mechanical stage vernier only reads down to 0·1 mm., or 100 microns. The size of objects down to 25 microns can be estimated with some accuracy, after a little practice, by comparing them with the diameter of the field (previously measured by means of the stage vernier) or with the travel, across the field, of a particle when the mechanical stage is moved through 0·1 mm.

Micrometer Eyepiece and Stage Micrometer.—For smaller objects, and accurate measurement, some form of eyepiece micrometer must be used.

Microscopic measurements are based on comparison methods. In other words, an arbitrary scale, lying in the focal plane of the eyelens so that its image is superimposed on that of the specimen, is compared first with a stage micrometer, for calibration, and then with the interval to be measured, care being taken that none of the adjustments are altered (beyond slight readjustment of the focus) when the specimen slide is substituted for the stage micrometer. The degree of magnification will depend on the size of the object; it will be easier to measure if it is considerably magnified provided that a sharp and well-defined image is obtained. Since the method is a comparison method, however, the magnification itself is immaterial and its value need not be known.

Stage Micrometer.—The stage micrometer usually consists of two millimetres divided into tenths and hundredths, or of one millimetre divided into tenths, the last tenth being subdivided into hundredths. Stage micrometers can also be supplied divided into hundredths and thousandths of an inch.

It will often be found that the intervals between the smaller divisions are unequal. This may lead to serious errors when measuring very small objects. It is always advisable to check this by projecting a highly magnified image of the micrometer on to a glass screen, or a sheet of white paper, and measuring the intervals between the divisions with a pair of dividers. The results should be carefully noted and the same divisions used for measuring very small particles, i.e. the ones which most nearly approximate to the average value found.

Procedure.—The arbitrary scale in the micrometer eyepiece is first calibrated by observing the number of divisions on the scale contained in one or more of the larger or smaller divisions of the stage micrometer, according to the degree of magnification. For convenience, the length of the drawtube may be slightly adjusted so that the result is a whole number. If, for instance, five divisions on the eyepiece micrometer are slightly more than one division on the stage micrometer, a slight readjustment of the drawtube will make these intervals exactly equal. This adjustment must not exceed certain limits, or the quality of the image will deteriorate.

In the example chosen above, if the single division on the stage micrometer is 0·01 mm., then each division on the eyepiece scale will be equal to $0{\cdot}01/5 = 0{\cdot}002$ mm., or 2 microns.

If the specimen slide be now substituted and the object to be measured is observed to span 7·5 divisions on the eyepiece scale, its actual size will be: $7{\cdot}5 \times 2 = 15$ microns.

Calibration.—It is often recommended that a micrometer scale be used that can be dropped into any eyepiece, and that calibrations should be made and noted once and for all, for every combination of eyepiece and objective. This is not good practice. When measuring very small objects, every care must be taken to secure a clearly defined image. This entails careful adjustment of the drawtube or objective collar, and the initial magnification of the objective is correspondingly altered. The only sound practice is to calibrate with the stage micrometer every time a measurement is made (or a set of measurements under identical conditions).

Photographic Method.—For very accurate results, a good plan is to take a photomicrograph and then, removing the specimen and substituting the stage micrometer, to measure one or more divisions on the focusing screen with a pair of dividers. As soon as the negative has been processed, the dimension measured by means of the dividers can be marked out on the negative by means of two vertical scratches.

Any portion of the specimen on the photograph can then be accurately measured with a finely divided ruler, using the distance between the vertical marks as a reference scale. Any enlargement subsequently made will, of course, correspondingly enlarge the scale.

Millimetre Eyepiece Scale.—The scale in the micrometer eyepiece actually measures the primary image formed by the object glass in the focal plane of the eyelens. If, therefore, the eyepiece

scale is divided into millimetres instead of arbitrary divisions, the size of the primary image of any object can be read off in millimetres. The *actual* size of the object will be equal to this value divided by the initial magnification of the objective. The value of the initial magnification can be accurately measured, for a given tubelength, by substituting a stage micrometer for the specimen slide. This principle is applied in the Beck micrometer eyepiece which is of the Ramsden type (focus outside the lenses). On the left is a vertical scale divided into

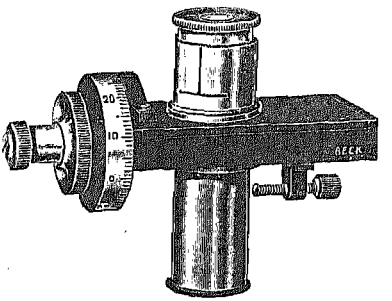


FIG. 34.—Micrometer eyepiece (Beck).

half-millimetres, and on the right a vernier or slanting scale reading to 0·1 mm. The image of the object shown on the diagram measures 3·25 mm. If the initial magnification of the objective is found to be 50 diameters, then the actual size of the object is $\frac{3{\cdot}25}{50} = 0{\cdot}065$ mm. This is a useful device, and the eyepiece is further provided with a focusing device so that the scale can be sharply focused in the field of view.

Focusing Eyepiece.—The focusing feature is incorporated in the better types of micrometer eyepieces. It is a great convenience since observers with abnormal sight may find it difficult or impossible to focus the scale sharply unless some form of adjustment is provided. If the scale is not in sharp focus, measurements are bound to be inaccurate.

When using any of the above methods the correct procedure, whenever possible, is to take as many measurements as possible and then work out an average. This does not, of course, apply to single items, but it is very important in the case of pollen grains, fungus spores, minute protozoa, or bacteria, etc. In such cases

there will be slight variations between individuals in addition to errors of measurement and the general plan adopted, number of individuals measured, and averages taken must depend on the nature of the investigations and their final purpose.

Filar Eyepiece.—The filar eyepiece is used for work requiring a greater degree of accuracy. The principle is exactly the same, but the divisions of the eyepiece scale are further subdivided by means of a hair-line set vertically across the field, which can be moved along the scale by means of a very fine screw carrying a graduated drum of large diameter. The drum is usually divided into 100 parts, so that, if one complete turn moves the hair across one division of the eyepiece scale, a hundredth of a division can be read off the drum (Fig. 34).

The eyepiece is naturally rather heavy and bulky, and it is sometimes used as a separate unit, on an independent stand, adjusted so that its axis coincides with the optical axis of the instrument, but with an interval of the order of 1 mm. between its lower face, and the upper face of the drawtube. This avoids tremor, when the drum is operated, with very high magnifications. Naturally this arrangement involves a readjustment of the drawtube so as to obtain the correct overall tubelength.

The limitation, as regards accuracy, with this type of micrometer lies in the quality and definition of the image and, above all, in its freedom from diffraction phenomena.

Measurement of Depth or Thickness.—Occasionally it may be necessary to measure the thickness of a transparent object or section. This form of measurement demands considerable skill, and great accuracy cannot be expected. The principle is to focus some detail on the upper surface of the object, and then some detail on the lower surface. The vertical travel of the objective is then measured by means of the graduated fine adjustment. It is necessary to use an object glass of fairly high power, at least 4 mm., and the aperture should not be less than 0·85 *NA*, so that the two planes of focus are sharply defined.

It is necessary to know the value of the scale divisions on the fine adjustment. The maker can usually supply this information, or the fine adjustment can be calibrated by measuring objects of known thickness, such as the edge of a previously gauged cover-slip.

The distance of travel as shown on the fine focus scale only represents the actual thickness of the object when the mounting

medium is air. In order to find the true thickness the movement of the fine adjustment must be multiplied by the refractive index of the medium in which the object is immersed, that is, the medium between the two planes of focus.

Variations from the assumed refractive index of the medium introduce an error, but this error is considerably less than that introduced when determining the exact planes of the upper and lower surfaces.

COUNTING

Making counts by means of the microscope is an important technique with many applications. Counts can be made without the use of any special apparatus, but unless such work is only occasional, it pays to procure the requisite accessories.

As an example of counts which can be made without special apparatus, mention can be made of a method of estimating the number of bacteria in a given volume of milk. These are first stained blue by means of Newman's stain, a given volume of the sample, say 0·01 c.c., being spread evenly over a 1-cm.^{2} cover-slip and allowed to evaporate to dryness.

The area of the field of view is then determined by measuring its diameter with a stage micrometer. The milk smear is then focused, and the bacteria, visible as bright blue particles, counted within the field of view. The slide is then shifted and the process repeated for a number of fields, the average of all counts being taken.

If n is the average number of organisms counted, and N is the number of organisms in 0·01 c.c., then:

$$N = \frac{\text{Area of film}}{\text{Area of field}} \times n = \frac{1 \times n}{\text{Area of field in cm.}^2}$$

The number of organisms per 100 c.c. of milk would then be $10{,}000 \times N$.

This method can be used for many similar applications, particularly when the particles are so small that a thick layer of medium cannot be used on account of focusing difficulties. In such cases the substance must be examined as a flat film, or smear, and the determination of the total number of particles per unit of volume reduced to a ratio of *surfaces*. From this point of view the area of the field is a useful standard for counting. If the organisms or particles are very numerous, the area of the field

can be reduced by using an objective of higher power. When this is inconvenient, or if the particles are still too numerous for counting, a graticule, or glass disc divided into squares of suitable size, can be placed in the focal plane of the eyelens. One or more of these squares can then be used instead of the whole field as the standard area for counting. If the actual area of one of these squares is S and the initial magnification of the objective M diameters, then the area of the smear over which the count is made will be:

$$\frac{S}{M^2}.$$

If the square chosen is 5 mm. each way, for example, its area is 25 mm.^{2} If the initial magnification of the objective is 50×, then the actual smear area to which the count applies is

$$\frac{25}{50 \times 50} = 0{\cdot}01 \text{ mm.}^2$$

If the smear as a whole has an area of 1 cm.^{2}=100 mm.^{2}, then the ratio of areas is

$$\frac{100}{0{\cdot}01} = 10{,}000.$$

If the average number of particles counted per unit square is 15, for example, then the total number of particles in the smear will be 10,000×15=150,000. Since the smear was made from a definite volume of liquid, the number of organisms or particles in any given volume can be easily estimated.[1]

Counting Chambers.—These are the most generally used for counting the number of particles or minute organisms contained in a given volume of fluid.

A circular groove is cut in the thick glass slide (Fig. 35) and the inner circular surface is then accurately ground down until its surface is 0·1 mm. below the level of the plate of glass. This portion is also accurately ruled into squares of known area, subdivided for convenience into smaller squares.

A drop of liquid containing the particles to be counted is placed on this surface, and a cover glass is placed over the cell.

It is easy to see that if the surface of one square is 0·1 sq. mm., for example, then the volume of liquid over that square is

[1] Some makers supply special eyepieces fitted with a diaphragm in the focal plane of the eyelens. This diaphragm has a *square* opening which can be adjusted to different values marked on a graduated scale.

$0{\cdot}1 \times 0{\cdot}1 = 0{\cdot}01$ cubic mm. If the average number of particles counted over several identical squares is 25, then there are 25 particles in every 0·01 cubic mm. of liquid, or 2,500 particles per cubic millimetre.

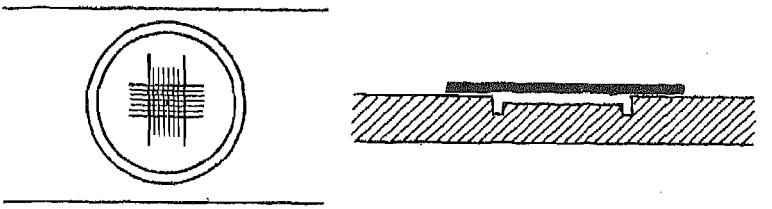


FIG. 35.

More generally, if the surface over which the count is made is s, and the depth of the cell is d, the volume is $d \times s$. If the number counted over the square is n, then the number in any volume V of the liquid will be

$$\frac{V \times n}{d \times s}.$$

Estimating Weights.—If we require to determine the weight of such minute objects as pollen grains, fungus spores, etc., we can proceed as follows:

Taking a weight, w, of the particles, we mix it thoroughly with a fairly large volume of some suitable liquid such as glycerine, or olive oil. Let this volume be V.

We now count the number of particles contained in the volume $d \times s$ as in the preceding method. The number of particles in the total volume V is thus:

$$\frac{V}{d \times s} \times n.$$

But this is also the number contained in the weight, w.

Thus the weight of a single particle would be

$$\frac{w}{\frac{V \times n}{d \times s}} = \frac{w \times d \times s}{V \times n}.$$

It is more usual, however, to give the number of particles per milligram.

As an example of this method let us suppose that we weigh out 5 milligrams of the particles and dilute in 10 cubic centimetres of liquid.

$$w = 5 \text{ milligrams.} \quad V = 10 \text{ c.c.}$$

If the counting square has an area $s = 1$ square mm. and the depth of the cell is 0·1 mm.,

$$\text{then } d \times s = 0{\cdot}1 \text{ cubic millimetres}$$

$$\text{and } \frac{V}{d \times s} = \frac{10 \times 1{,}000}{0{\cdot}1} = 100{,}000.$$

If we counted 5 particles over the square, $n = 5$, then there will be 500,000 particles in the original 5 milligrams, or 100,000 particles per milligram.

Another Method.—The weight of fine particles can be determined by another method which does not require any special apparatus.

The spores of *Lycopodium clavatum* L. are extraordinarily constant in size and weight and are known to number 94,000 to 1 milligram.

If we take equal weights of these spores and of the particles to be weighed and make an emulsion of the mixture in olive oil, we can examine a minute drop of this emulsion under the microscope. Taking a number of counts we can determine the number of particles present per 100 Lycopodium spores. Let this number be n, and let x be the number of particles per milligram, then:

$$\frac{n}{x} = \frac{100}{94{,}000} \text{ and } x = 940\, n.$$

Haemacytometers.—The haemacytometer is an apparatus for counting the blood corpuscles, and the method is a good example of counting methods generally since it also embodies a dilution process which can be usefully applied in many other cases.

The counting chambers used (Fig. 36) are made in one piece of glass and are usually of the transverse or open cell type, a form which has generally superseded the circular or closed cell owing to the ease with which liquids can be introduced into the cell by capillary attraction. The closed cell, on the other hand, prevents evaporation of the liquid, and this may be important in some cases.

The blood is first diluted with Toisson's solution, for either red or white corpuscles, or with a solution of acetic acid when a count of white cells only is being made.

In counting red corpuscles, a 1–200 dilution is used, and for white corpuscles, a dilution of 1–10. Specially graduated mixing pipettes are supplied for this purpose.

A drop of the mixture is placed in the centre of the counting chamber and the cover glass is then placed over the cell. In the Thoma haemacytometer the surface of the cell is ruled into squares one-four-hundredth of a square mm. each in area. Thus the amount

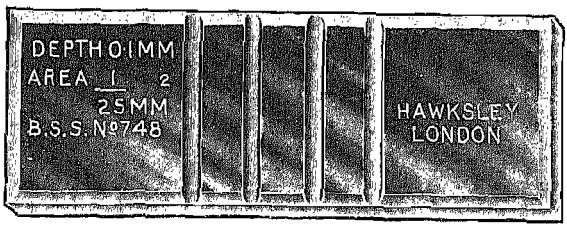


FIG. 36.—Haemacytometer counting chambers (Hawksley).

of liquid resting upon each square has a cubic capacity of one-four-thousandth of a cubic mm. (the depth of the cell being the standard 0·1 mm.). The liquid is allowed to settle, and the corpuscles will therefore be in contact with the bottom of the cell. It is now a simple matter to count the corpuscles in one square. It is advisable to count 100 squares, evenly distributed over the ruled area, and this is facilitated by the squares being framed off into blocks by means of triple rulings.

Having counted 100 squares, divide the sum by 100 (the number of squares counted) to obtain the average per square. Multiply by the dilution and divide by 1/4000 (the cubic capacity over one square).

The following formula can be applied:

$$\frac{D \times N}{S \times K}$$

where D = rate of dilution.
N = number counted (total over all squares).
K = cubic capacity over one square in cubic mm.
S = number of squares counted.

Example

D = 100 (dilution 1–100).
N = 1,350 (total number counted over 100 squares).
K = 1/4000 (square area 1/400 sq. mm. Depth 0·1 mm.).
S = 100 (number of squares counted).

$\frac{10 \times 1{,}350 \times 4{,}000}{100} = 5{,}400{,}000$ red corpuscles in 1 cubic millimetre of undiluted blood.

In the case of white corpuscles, as there are far fewer of these, the method generally employed is to count the total number over the whole ruled area of the counting chamber which is 1 sq. mm. In the above formula K would now be $\frac{1}{10}$ cubic mm. ($1 \times 1 \times 0{\cdot}1$), and D would be 10.

A mechanical stage should, if possible, be used. Illumination is important, or the rulings may not be easily seen. A $\frac{1}{6}$-inch objective is the most suitable for the purpose, but a $\frac{2}{3}$-inch can be used with a 25× eyepiece.

There are other forms of counting chambers, such as the Bürker, Füchs-Rosenthal, Brener, Neubauer, Zappert, etc. The method is the same, but the rulings, and hence the counting, differ.

More recently a differential blood plate has been introduced by Messrs. Busch which consists of a number of very fine parallel rulings instead of squares. The corpuscles are sucked down by capillarity and appear clearly and distinctly between the lines. They are counted along one line after another by means of the mechanical stage. Greater accuracy is claimed.

ULTRA-MICROSCOPIC METHODS

The ultra-microscope does not, of course, reveal any detail which cannot be revealed by the microscope. Its purpose is to reveal the presence of very minute particles which would be otherwise invisible. There is theoretically no limit to the smallness of a particle which can be rendered visible against a dark background by intense illumination. This will give no indication of its real size or structure, and the appearance of the particle is merely that of its diffraction image.

The principle is to suspend the particles in a liquid and to concentrate upon them a very narrow and intense beam of light accurately focused in a plane coincident with the axis of the microscope (the beam being at right angles to this axis). The

particles lying in this plane reflect and scatter some of the light, and their presence is revealed.

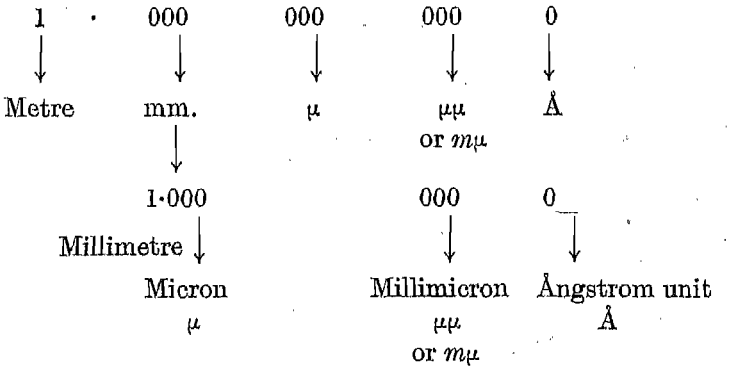
The size of such particles can be measured by an interference method due to Gerhardt. It is thus possible to measure particles the dimensions of which are only half the size of those which an objective would resolve under ordinary conditions. The method is beyond the scope of this chapter, and its applications restricted to very special fields, particularly the study of colloids.

Locating Objects.—A brief description of the Maltwood finder is included here since it bears some relationship to the methods we have described and the general use of rulings, scales, and graticules.

It is a standard 3×1-inch glass slide with a photographed network of squares, each square bearing a serial number. There must be provided some means of setting both the finder and the specimen slide in a fixed position on the stage. This may be done by referring both to standard readings on the mechanical stage scales, or reference marks may be made on the surface of the stage itself to ensure correct registration.

The object to be recorded is first brought exactly to the centre of the field of view. The slide is then removed, and the finder substituted. A numbered square will then be seen in the centre of the field, and this number is noted. When it is again required to examine the object, the finder is first located so that the number is in the centre of the field, then the slide is substituted in the same position and the object will be found.

Units of Measurement and Conversion Factors.—The following data may be useful to the reader, though more complete tables are given in standard books:



1 micron	= 1μ=0·001 mm.
1 μμ	= 0·001 μ
1 A°	= 0·1 μμ=0·0001 μ
1 A°	= a 250-millionth of an inch *approximately*
1 μμ	= a 25-millionth of an inch *approximately*
1 μ	= a 25-thousandth of an inch *approximately*
1 μ	= 0·00003937 inch
1 mm.	= 0·03937 inch
1/1000 inch	= 25·399772 μ
5,000 lines per inch	= 197 lines per mm.
1 line per μ	= 25399·77 lines per inch
1 square μ	= 0·00155 square thousandth of an inch.

CHAPTER IX

FILTERS

EXPERT photographers will tell you that the filter makes the picture. In the case of photomicrography this is so true that one cannot hope to obtain satisfactory results unless the use of colour screens or filters is properly understood. It is not, however, a question of photomicrography alone. Filters are of great assistance for ordinary visual observation and, partly for this reason and partly on account of the general importance of the subject, it is included in this special chapter as a complement to visual, and an introduction to photographic microscopy.

To understand the action and uses of filters it is necessary to consider the nature of light and colour and to compare the sensitivity of the eye to certain colours and under different lighting conditions, with that of the photographic plate.

VISIBILITY

Given a source of light, objects become visible through their greater or lesser ability to reflect light, and thus stimulate sensation in the eyes of the observer. An object which reflected no light would be invisible no matter how powerful the illuminant. It would represent a perfect black, and there are no such entirely non-reflecting objects known.

Every object thus has the power of reflecting towards the observer *some portion* of the light it receives from the illuminant.

Reflecting power, or the percentage of incident light reflected back to the observer's eye is thus, as a first approximation, a measure of the visibility and brightness of an object.

The most complete black reflects 1 to 2 per cent of incident light.

An almost perfect white (fresh snow), 70 to 80 per cent.

Intermediate tones (dark greys), 10 to 20 per cent; (light greys), 60 to 70 per cent.

The balance of the unreflected light is absorbed by the object, and lost as light.

COLOUR

So far we have dealt only with reflecting power regardless of colour. It is a well-known fact that a beam of white light entering

a prism emerges as a composite beam split up into the colours of the rainbow. If this composite beam is passed through a second prism, the colours re-combine into a single beam of white light. White light is thus a mixture of light rays of different wavelengths which, when blended, produce on the observer's eye a sensation of white. These colours gradually merge into one another; there is no sharp dividing line. Below the red are the infra-red rays invisible to the human eye and merging into radiant heat. At the other end of the scale come rays of shorter wavelength, the ultra-violet, which are also invisible. Photographic emulsions can be made sensitive to both infra-red and ultra-violet.

Colours of the Spectrum.

(Infra-red—longer than 730 $m\mu$)

Red	730–650
Orange	650–590
Yellow	590–575
Yellow-green	575–550
Green	550–510
Blue-green	510–490
Blue	490–440
Violet	440–400

(Ultra-violet—shorter than 400 $m\mu$)

Note.—1 $m\mu = 0{\cdot}001\ \mu$ (1 $\mu = {\cdot}001$ mm.

Primary Colours.—Experiment shows that pure white light can be obtained by mixing beams of blue, green, and red light only. These three simple colours can be so blended as to produce any other colour at will. For these reasons they are known as the *three primary colours*.

White light can thus be defined as light which gives rise to a sensation of white, and which is a blend, in equal proportions, of blue, green, and red light.

A non-coloured object reflects or absorbs every one of the constituents of white light *in equal proportions*. A grey object is grey, for instance, because, while it may only reflect some 60 per cent of the incident light, this reflection (and the absorption of the balance) is distributed evenly between the blue, the green, and the red rays. The reflected light is thus the same in *quality* as the incident light, but not in *quantity*.

Colour results from an alteration in *quality* of the reflected light, that is, from an alteration in the *proportions* of the primary

colours in the reflected beam. A coloured object appears coloured because, instead of reflecting (or absorbing) the three primary colours in equal proportions, it reflects some and absorbs others. Such an object may absorb the whole of the green and red, for instance, only reflecting the blue. It will then appear blue. A green object absorbs all the blue and red light. A purple colour results from the reflection of blue and some red, and the absorption of all the green. The object may absorb only one colour; yellow results from the total absorption of blue, and the reflection of green and red.

Thus: Visibility or brightness depends on the *quantity* reflected.
Colour depends on the *quality* of the light reflected.

Both effects are generally combined, giving an impression of both colour and brightness.

To make this clearer, the conclusions may be summarised as follows:

(1) If the *blue-green-red balance is unaffected* by reflection, the object is *colourless*.

It will appear white if it reflects 70 to 80 per cent.
It will appear grey if it reflects 10 to 60 per cent.
It will appear black if it reflects 1 to 5 per cent.

(2) If the *blue-green-red balance is affected* by reflection, the object appears *coloured*.

It will appear blue if it absorbs green and red.
It will appear green if it absorbs blue and red.
It will appear red if it absorbs blue and green.
It will appear yellow if it absorbs blue only.

The great bulk of yellow light in nature is a combination of green and red, but there does exist a very small amount of pure yellow light which cannot be split up into red and green. The quantity is very small.

Colour and Brightness.—There are two further points which require consideration.

(1) *The colours are not equally reflected.* Only approximately one-quarter of the blue light is reflected by a blue object, whereas a green object will reflect one-third of the light, and a red object as much as 75 per cent. It follows that, other things being equal, a red object would appear much brighter than a green object, and a blue object would be the dullest of the three.

This is not the case because, in addition to this:

(2) *The eye is not equally sensitive to all colours.* It is most sensitive to green, with a 66·6 per cent stimulation of the retina, then to red, with a 30 per cent stimulation, and least of all to blue, with 3·3 per cent approximately.

This can be shown graphically as follows (Fig. 37):

The net effect is that the brightest of the three colours is green, the sensitivity of the eye to this colour more than compensating for the fact that it is less strongly reflected than red. It is for this reason that achromatic object glasses are corrected for a yellow-green "preferred" colour which happens to be that to which the human eye is most sensitive.

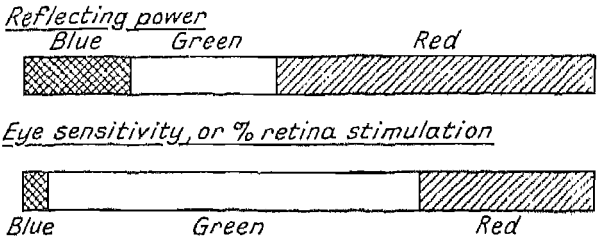


FIG. 37.

Of the other colours, yellow, which combines the advantages of green and red, will appear by far the brightest.

Colour of Transparent Objects.—The same principles apply in the case of transparent objects viewed by transmitted light. A red glass, for instance, will pass red rays and stop all green and blue light. A yellow glass absorbs the blue, but passes both green and red.

It is thus possible by means of transparent coloured screens or filters to illuminate an object with light of any required colour.

COLOURED LIGHT

We have so far considered the effects obtained with white light defined as composed of equal proportions of blue, green, and red light. The use of coloured light to illuminate an object will naturally affect the quality of the reflected beam profoundly.

A blue object absorbs green and red light. If either of these is used as an illuminant, the whole of the light will be absorbed, and the object will appear black.

In the case of visual observation with the microscope we seek

to obtain contrast of the object against its background, or contrast within the object itself to reveal detail.

If the object is naturally coloured, or if it has been stained, much can be done to accentuate contrast or reveal detail by the use of suitable filters.

CONTRAST AND DETAIL

In the case of photomicrography we are interpreting differences of brightness and colour in terms of shades of grey varying from deep black to almost pure white, but otherwise the same contrast and detail desiderata apply. We can no longer rely on colour contrast, however, and such contrasts must be interpreted in monotone.

The amount of light reflected or transmitted by the object would determine the relative values of such tones precisely were it not for the fact that both the eye and the photographic plate respond unequally to light of different colours, and that this inequality of response is entirely different in the case of the human eye and in the case of the photographic plate.

Clearly, therefore, we must have some method of control or compensation so as to obtain an accurate interpretation of the colour values in terms of blacks, greys, and whites on the photograph.

This is what the landscape photographer seeks to achieve by means of filters.

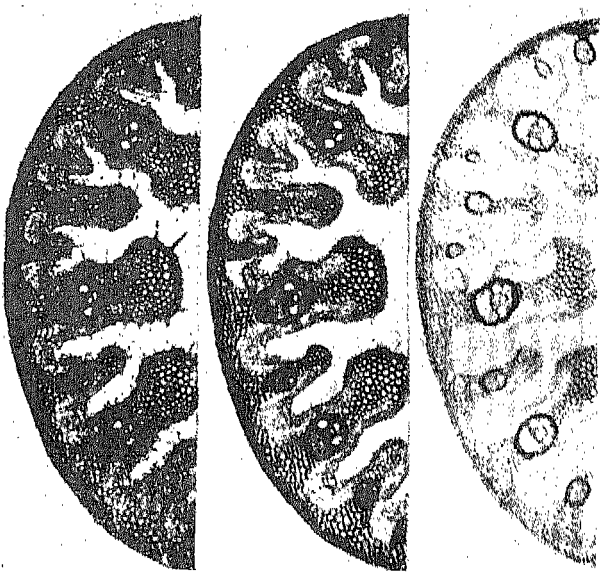
USE OF FILTERS

When looking at white clouds in a blue sky the observer is conscious of sharp contrast, because the eye, being insensitive to blue, is very little stimulated by the background which thus appears dark, while it is strongly stimulated by the white light from the clouds, owing mainly to its green and red components.

If such a sky is photographed, the result will be entirely different. The ordinary photographic emulsion is highly sensitive to blue, but quite insensitive to either red or green. It will be strongly affected by the blue background which will appear light, and it will react about equally to the blue component from the white clouds. It follows that all contrast will be lost and the clouds and sky will be reproduced as an almost uniform and very light grey tone.

For correct interpretation of these colour values, a photographer

will use panchromatic material which is sensitive to the green and red components of white light, but is still much too sensitive to blue. He will correct this defect by interposing a yellow filter of suitable intensity which will transmit both red and green, but will stop most of the violet and blue. The clouds will then stand out in very light grey or white against a dark background.



500–540 570 640

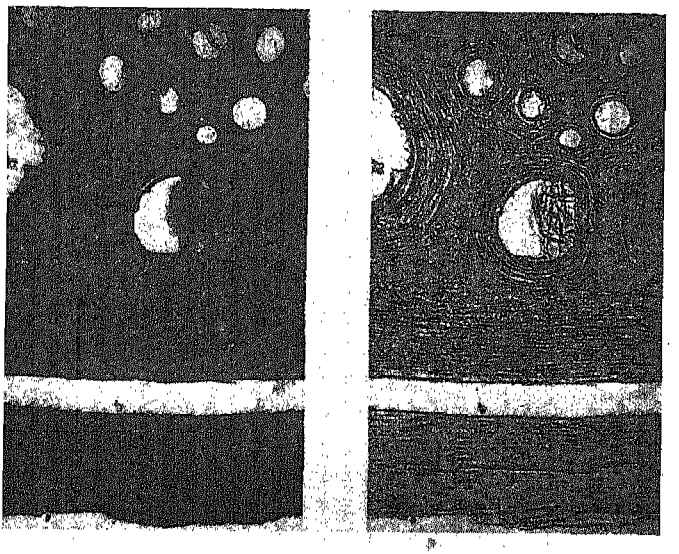
FIG. 38.—Eosine stained section. (Kodak, Ltd.)

The microscopist uses his filters for a rather different purpose. He is not so much concerned with correct interpretation as with a deliberate distortion of these relative values which will result in the accentuation of contrast against the background, or within the specimen itself.

He works on the principle that the contrast presented by a coloured object can be entirely controlled by altering the colour of the light by which it is examined or photographed.

A useful example is given by the photomicrographs of a section stained with eosine reproduced in Fig. 38 (Kodak Ltd., Wratten Division). The section appears pink, eosine absorbing light from 490 to 530 $m\mu$. If the section is photographed by light of 500–540,

completely absorbed by eosine, the section is *entirely black*, the maximum amount of contrast being obtained. Owing to this excess of contrast, the detail of the section is indistinguishable. Photographing at 570, on the border of the absorption band, we get a greatly lessened contrast which, for this particular section, gives the best result. If the section is photographed by red light,



Blue filter Red filter

FIG. 39.—Whalebone section (Kodak, Ltd.).

640, which is completely transmitted by the section, the contrast entirely disappears and the result is flat and useless.

To reveal detail, or to obtain contrast within the object itself, instead of contrast of the object as a whole against its background, the specimen must be illuminated with *light of its own colour*, or light which it will reflect or transmit.

A good example of this is the whalebone section reproduced in Fig. 39; the first photograph, taken with a blue filter, gives maximum contrast for the specimen as a whole, but no detail whatsoever.

The second photograph, taken with a red filter, shows maximum detail within the object itself.

The secret of success is to obtain satisfactory balance between what might be termed external and internal contrast, bearing in

mind that the former results from illumination by a colour absorbed by the specimen, whereas the latter results from illumination by a colour transmitted by the specimen.

Many photomicrographs result in dull negatives completely lacking in both contrast and fine detail. The reason for this is that the blue portions photograph white, but the red portions stained with eosine, fuchsin, etc., contain so much blue that they also photograph white so that the resulting picture is entirely *flat*.

In the case of differential staining, red particles against a green background, for instance, there are two possibilities. A green filter will result in the appearance of black particles on a light background, while a red filter will show up the particles as light specks on a dark background.

Knowing the degree of sensitivity of the photographic emulsion used to light of different wavelengths, and taking into account the colour or colours of the object itself and its background, we can select a filter which will give a beam of light emerging from the object of precisely the right quality to reveal detail or accentuate contrast exactly as and where required.

(1) If a colour is to be rendered as black as possible, then it must be viewed or photographed, by light which is completely *absorbed* by that colour; that is, by light of the wavelengths comprised within its absorption band. If this is carried too far, all detail will be lost.

(2) If contrast is required, not against the background but within the object itself, the specimen should be viewed or photographed *by the light which it transmits*. The class of microscopic objects which best respond to this procedure is that of the usual insect preparations which, when photographed by yellow or red light, will give very satisfactory results. If this is carried too far, the whole object will merge into the background.

The best method of determining the contrast required by any object is to examine it visually first by means of a combination of filters transmitting as completely as possible light of the wavelength absorbed by the preparation. This will give maximum contrast and minimum detail. Other filters can then be tried transmitting light less completely absorbed until the degree of contrast, and the balance between contrast and detail, are satisfactory to the eye.

The Photographic Emulsion.—If colour filters are used for

the illumination of an object, photographs cannot obviously be taken on ordinary plates, which are sensitive only to blue and violet light. It has already been stated that the response of a photographic plate to different colours is not at all the same as that of the eye. The following diagram makes this plain (Fig. 40).

Orthochromatic plates are sensitive to yellow-green, but their use narrows down work to only a small band of the yellow-green region in addition to blue and violet. In order to make full use of the possibility of contrast control afforded by filters, panchromatic material must be used.

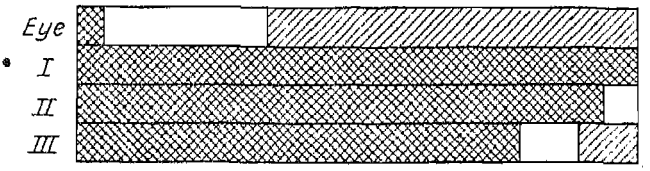


FIG. 40.—Relative sensitivities.

I. Ordinary plates. Violet-blue, 100. Yellow-green, 0. Red, 0
II. Orthochromatic. Violet-blue, 95. Yellow-green, 5. Red, 0
III. Panchromatic. Violet-blue, 80. Yellow-green, 10. Red, 10

For maximum resolution, however, the use of blue or violet filters is indicated because the shorter the wavelength of the light, the greater the power of resolution of the instrument. The contrast factor ("Gamma") of panchromatic material falls in value as the violet is approached, and it will be almost impossible to obtain sufficient contrast with faintly visible unstained structures. Blue-violet sensitive "ordinary" emulsions of the process grade are excellent in such cases, and for such subjects as diatoms.

Summarising the above conclusions:

(1) For contrast: A filter which completely absorbs the colour of the object, i.e. a filter *complementary* to that colour.

(2) For detail: A filter of the same colour as the object.

(3) With contrast filters: Use a soft plate.

(4) With detail filters: Use a contrasty plate.

(3) will help to avoid unduly black and white negatives, whereas (4) will avoid unduly flat results.

FILTERS FOR CONTRAST
(Complementary colours)

Object	Filter for contrast
Red	Blue-green
Orange	Violet
Yellow	Blue
Pink	Deep green
Purple	Light green
Blue-green	Red
Green	Pink
Blue	Yellow
Deep blue	Orange
Violet	Orange

This is a sufficient basis for work of a general nature. For very exacting work, the exact shade of colour is of great importance. Manufacturers publish filter atlases which tabulate a very wide range of filters specifying the exact wavelengths which they transmit. Usually these tables give the transmission curves and percentage transmission at each 100 Ångstrom units (1 Ångstrom unit $=0{\cdot}1\ m\mu$).

For most purposes the set of nine special micro-filters supplied by Ilford Ltd. will suffice. Their particulars are given here as typical of such filters. Kodak Ltd. supply a wide range of filters known as the "M" Wratten filters.

Filter.	Colour.	Transmission Range. Å°	Uses.
Micro 1 No. 305	Blue-Violet	3,500–5,100	For obtaining the strongest contrast with yellow-stained specimens. For maximum resolving power in the photography of unstained specimens with fully colour corrected lenses.
Micro 2 No. 303	Blue	4,300–5,700	For increasing the contrast of yellowish or yellow-stained specimens. Useful for obtaining the maximum resolving power by visual inspection using fully colour corrected lenses.

Filter.	Colour.	Transmission Range. Å°	Uses.
Micro 3 No. 405	Green	4,800–6,100 6,800–infra-red	Used in photomicrography of histological specimens. For increasing contrast of red or purple stains. For maximum resolving power in photography and visual examination for lenses corrected for the yellow-green.
Micro 4 No. 110	Yellow	5,000–infra-red	For obtaining the greatest detail in photography of yellowish or yellow-stained specimens. For increasing the contrast of specimens stained violet or blue.
Micro 5 No. 202	Orange	5,500–infra-red	For increasing the general contrast of the negative when photographing transparent faintly coloured objects.
Micro 6 No. 501	Purple	3,500–4,900 6,400–infra-red	For increasing the contrast of specimens stained green or yellow-green.
Micro 7	Magenta	3,400–5,000 5,900–infra-red	For increasing the contrast of specimens stained green.
Micro 8 No. 104	Very pale yellow	Absorbs ultra-violet. Transmits all visible light with partial absorption in the blue.	For visual examination where it is desirable to reduce excessive contrast between insect preparations and the background.
Micro 9 No. 108	Pale yellow	6,600–infra-red	For slightly increasing the contrast of specimens stained blue or violet.

The corresponding Wratten filters are approximately as follows:

Ilford Micro 1	Wratten 47 C–5
2	45 H
3	58 B
4	15 G
5	22 E
6	35 D

Since filters are of special importance when working with stained specimens, the following table, giving the spectral absorptions of some biological stains, may prove a useful guide.

Stain.	*Absorption band.*
Acid Fuchsin	530–560
Aniline blue	550–620
Azure I	580–640
Basic Fuchsin	520–550
Bismark brown	General in blue
Carbol Thionine	550–600
Carmine	500–570
Congo red	480–530
Crystal violet	550–610
Eosin B	480–550
Eosin Y	490–530
Erythrosine	510–540
Gentian violet	570–600
Gram	General in green
Haematoxylin (Ehrlich)	Gradual through green
Harmatoxylin (Heidenhain)	560–600
Iodine green	620–650
Light green S.F.	600–660
Leishman	General in green
Methyl green	620–650
Methyl orange	430–500
Methyl violet	550–600
Methylene blue	600–620 and 650–680
Orange II	460–510
Phloxine	510–550
Picro-carmine	510–530 and 560–570
Rose Bengal	530–560
Safranin O	480–540
Sudan III, IV	General in blue and green with maximum at 500

Having decided on the effect it is required to produce, and knowing the absorption bands of the colours present in the preparation, it is a comparatively simple matter to select a filter or filters from the absorption data given by the manufacturers, always bearing in mind the nature of the photographic emulsion which is to be used.

It is naturally impossible to give instructions for all cases, and the best guide, as already stated, is the visual inspection of the specimen through the filters and the use of the combination with which the greatest contrast or the greatest detail is obtained.

As a general guide, when contrast is required, use—

A red filter for blue-stained preparations
A red filter for green-stained preparations
A green filter for red-stained preparations
A blue filter for yellow-stained preparations
A blue filter for brown-stained preparations
A green filter for purple-stained preparations
A yellow filter for violet-stained preparations.

The filters consist either of gelatine film stained with dyes selected for their brightness, sharpness of absorption, and stability, or of carefully selected and optically perfect glass. The gelatine films are usually cemented between two sheets of optical glass by means of Canada Balsam, the cementing both protecting the filters and rendering them more transparent. The proper position for the filter is between light-source and microscope and not between microscope and camera.

Compensating Filters for Achromatic Objectives.—We have seen that with an achromatic lens the yellow-green and pale blue images coincide, whereas the red, dark blue, and violet images lie in a different plane, and are therefore out of focus. Since the eye is very sensitive to green and yellow, and not nearly so sensitive to the remaining colours, this defect is hardly noticeable for visual observation.

The photographic emulsion, however, is particularly sensitive to deep blue and violet, that is, to those portions of the composite image which are out of focus. This will result in poor definition, and for this reason it is always preferable to use apochromatic objectives for photomicrography.

If achromatic objectives are used, the difficulty can be overcome by using a filter which will absorb the unwanted colours, in this

case a yellow-green filter. The more poorly the objective is corrected, or the more strongly the object is coloured, the deeper the colour of the filter. Such a filter will appreciably improve the performance of achromatic lenses for visual work as well, particularly with high magnification aiming at the resolution of fine detail. Ilford 3+4 or Wratten B+b are suitable combinations for this purpose (510–615, 540 dominant).

CHAPTER X

PHOTOMICROGRAPHY

THIS has become so much a part of everyday work with the microscope that a general outline of the principles involved is essential to the plan on which this book is based.

In effect, since the microscope can be made to give a real image which can be projected on to a screen, all that is necessary, to take a photograph, is to substitute a sensitive plate for the screen, and provide some form of light-proof enclosure. In photomicrography it is the microscope which matters. The camera is a mere box.

The apparatus can be divided into three distinct classes:

(*a*) Simple devices such as the eyepiece camera by means of which photographs can be taken easily and quickly for the purpose of recording successive phases of a process, stages of growth, etc., rather than for obtaining highly critical results revealing the finest structure.

(*b*) Equipment consisting of a long-extension camera and optical bench on which a standard microscope can be fitted in a horizontal or vertical position. This, when properly designed, is suitable for the most exacting work, where the use of highly specialised equipment is not otherwise justified.

(*c*) Elaborate photomicrographic equipments specially designed as complete units, in which the microscope and fittings are generally of unorthodox design. The combined unit is adapted for routine procedure in large laboratories and industrial research departments.

I propose to deal with the first two classes only. The third includes costly apparatus beyond the scope of this book, and detailed literature is available from the manufacturers.

We will first consider certain fundamental principles.

I. FUNDAMENTAL PRINCIPLES

The fundamental principles of microscopy naturally apply to photomicrography, but there are some additional factors which need consideration.

(1) We are dealing with a real image, instead of a virtual one.

(2) This image is projected on a screen, or on the plane of a thin sensitive emulsion coating.

The Real Image.—In order to obtain a real image, the primary image projected by the object glass must lie outside the principal focus of the eyelens. Theoretically, the correct procedure is to draw out the eyepiece very slightly after setting the instrument for correct visual focus. It is unsound to alter the setting of the object glass focus after it has been adjusted with all necessary corrections for visual observation. Under such conditions the primary image is formed at the exact conjugate focus for which the lens has been corrected. For this reason projection eyepieces have an adjustable eyelens which can be so set that the eyepiece diaphragm is sharply focused on the screen; when the primary image formed by the objective is in the plane of this diaphragm, the secondary image will be sharply focused on the screen.

In practice, unless such eyepieces are used, it is usual to bring about a slight change in the position of the primary image by a readjustment of the focus, instead of readjusting the eyepiece. There is little appreciable difference.

The Projected Image and Depth of Focus.—The projection of the image on a screen or sensitive plate introduces certain important restrictions.

The eye being absent, there is no accommodation, and the photographic plate will only record what is in sharp focus in its plane at one time. As a result of this, the depth of focus becomes a factor of great importance, if the photograph is to be clear, sharp, and *intelligible.*

The depth of focus is inversely proportional to:

(*a*) The numerical aperture.

(*b*) The degree of magnification.

For a given objective the depth of focus has a definite value in the plane of the eyepiece diaphragm where the primary image is formed, depending on the numerical aperture and the initial magnification for which the objective has been computed, these two values being linked and together characteristic of that objective.

Further magnification beyond this point may be obtained by eyepiecing or extending the camera. Whichever form is used, or whatever combination of these forms, the depth of focus will

decrease as the magnification increases. Points in planes other than the plane of sharpest focus are represented on the image as "circles of confusion". So long as these are small enough to be perceived as points by the eye, they will still appear in focus. Increasing the size of the image will also increase the size of these circles of confusion. This means that if we wish to increase the magnification while retaining a sharp image, we must start with circles of smaller diameter or, in other words, the corresponding points must lie in planes *closer* to the sharp focus plane and hence, the depth of focus is reduced.

Photographic enlargement from the negative cannot affect depth of focus in this sense, because we are enlarging a plane surface, and the ratio between the sizes of the anti-points corresponding to the plane of sharpest focus, and the larger circles of confusion from points in other planes, is now fixed. Nevertheless those portions of the negative which correspond to points outside the plane of sharpest focus, and are represented by circles of confusion of some magnitude, will rapidly cease to be perceived as points and cause blur when enlarged, so that no real advantage is gained in this way.

There is, however, a considerable gain if an objective of lower *NA* is used. For equal overall magnifications, the depth of focus is inversely proportional to the numerical aperture.

It is an advantage to use eyepieces of comparatively low power, and to compensate for this by extending the camera beyond ten inches. Long camera extensions, however, are very inconvenient and lead to tremor and loss of brilliancy so that, where considerable magnification is required for any reason, it is an advantage to obtain part of the magnification by enlargement from the negative.

Magnification, Emulsion Resolution, and Grain.—In the case of visual observation, we are concerned only with the ability of the objective to resolve fine detail. Sufficient magnification must then be provided for the smallest interval resolved to be enlarged so that it is comfortably seen by the eye.

In the case of photomicrography there are really two stages of resolution: one by the objective, and one by the sensitive emulsion. Here, the smallest interval resolved by the objective must be sufficiently magnified to ensure that it will be safely resolved by the emulsion. If this is not the case, the detail, though resolved by the microscope, will be lost on the negative, and no amount of photographic enlargement from the negative will reveal it.

This is an example of empty magnification by photographic enlargement precisely similar to that caused by high eyepiecing with an objective of insufficient numerical aperture.

Nevertheless, photographic enlargement from the negative is important. In photographic work very considerable magnification is often used, and the quality of the apochromats is such that such magnification is possible. Long camera extensions, as we have seen, are inadvisable, so that photographic enlargement is the best method in such cases.

How much can we rely on enlargement from the negative? We must express this differently; it is really a matter of how little magnification can be safely used with any given emulsion. These considerations apply particularly to eyepiece and miniature cameras. Their length is fixed, and usually only a fraction of 10 inches, so that the magnification may be relatively very small.

With very fine grain emulsions designed for the highest resolution, there is really no practical minimum to worry about, but these are not always indicated, and this is only true of these very special emulsions. Generally speaking, we shall be using ordinary or process plates, or commercial panchromatic material, so that the grain and minimum magnification are points which must be taken into account.

Grain.—The light sensitive coating consists of a suspension of crystalline grains of silver halide in gelatine. This is the emulsion.

The silver halides are either silver chloride, silver iodide, or silver bromide, the latter being the main constituent of negative emulsions. These grains are very small, but they vary in size over a wide range, from about one-thirtieth of a micron in Lipmann emulsion to as much as 5 microns in some high-speed emulsions.[1] The average grain size of a fast commercial emulsion is about 1 micron. The greater the average size of the grains, the more sensitive the emulsion, or the "faster" the material; the greater the uniformity of the grains, the more contrast the emulsion will give.

Consequently "speed" is incompatible with fine grain, though modern methods have gone a long way towards reconciling these factors.

This would be quite simple, and the size of the grains as given above would hardly interfere with resolutions were it not for various other phenomena, such as "clumping", infection, and light scatter.

[1] H. Baines, Ass. for Scientific Photography, 1945.

(1) The grains, whatever their average size, may not be evenly distributed within the emulsion. This is one of the main manufacturing problems, particularly with very fine grains. In some cases a form of supersonic vibration has been applied to the emulsions to bring about a more uniform distribution. The grains tend to gather into groups or clusters, in contact with one another. These develop as a single unit even though one constituent crystal only has been affected by light. The presence of these clusters leads to coarse grain.

(2) During the process of development, the grains increase in size, and if a developing grain touches other crystals, the developing process will spread to them as well so that a grain cluster results which is much larger than the original grain. This phenomenon, known as infection, is unfortunately increased by the use of the more powerful sensitising chemicals so that here again fast material is incompatible with fine grain.

(3) Another limiting factor is the degree of light-scatter within the emulsion.

The more deeply the light penetrates into the emulsion, the greater the light-scatter. The greater the exposure, the deeper the light will penetrate, so that the image must be kept thin and near the surface of the emulsion for maximum resolution.

Modern photographic plates have a very considerable exposure latitude, and this fact is often taken advantage of by the photographer owing to the difficulty of correctly judging exposure times in photomicrography. He will deliberately over-expose. But this latitude is obtained by the super-position of different emulsions of varying sensitivity, so that an over-exposed image lies very deep, and this is incompatible with maximum resolution.

In recent years new emulsions have been made which are extremely thin (of the order of 8μ). They can be provided with a protective coating of clear gelatine which does not appreciably affect resolution. There is little doubt that this material is ideal for the most exacting work requiring extreme resolution. Before the outbreak of war such material was not generally available except as 35-mm. celluloid film.

These difficulties can be partly overcome by:

(*a*) Using the finest grain emulsions, which means sacrificing speed. Speed is of little value for most photomicrographic work.

(*b*) Using a fine grain developer to reduce grain enlargement and infection.

(*c*) Avoiding a longer exposure than is strictly necessary, so as to reduce light-scatter.

A fine grain developer reduces the growth of the grain to a minimum and checks it at a sufficiently early stage to prevent infection spreading. It has an excess of sodium sulphite which, with a slow developer, will dissolve away the affected surfaces of the infected grains before the developer has time to reduce them to silver. Those grains which have been affected directly by light are affected in depth and will therefore develop normally.

Such developers contain paraphenylene-diamine. They are Sease I, II, or III, meritol, M.C.M. 100, Kodatol, Metol-thio-cyanate, etc.

Maximum Magnification.—So much for grain. It now remains to be seen how its presence affects resolution by the emulsion, and consequently what the minimum magnification must be, prior to the negative stage, in order to ensure complete resolution of the detail already resolved by the object glass.

For photographic work, it is preferable to use low-power eyepieces. The special photographic or projection eyepieces, which give an extremely sharp image, may have such low magnifications as 2 or 3 diameters. Otherwise 4× or 6× eyepieces (with an 8× or exceptionally a 10× eyepiece for high magnification) are most generally used.

This is sufficient to give the maximum magnification compatible with the numerical aperture and revealing the whole of the detail resolved. We have also an additional means of increasing the magnification by means of the camera extension. This, assuming an extension up to 25 or 30 inches, gives an additional factor up to 3× enabling us to use an eyepiece of lower power for a given overall magnification.

Now the criterion is no longer the smallest interval which the eye can clearly perceive, but the average probable size of the emulsion grain taking into account clumping and infection. There is therefore a minimum overall magnification. Most authorities fix this value at about 600× the numerical aperture, but this is a very safe figure and can certainly be considerably reduced when using special emulsions and superfine grain developers.

The resolution of a photographic emulsion is a complex phenomenon, but it may be taken to range from 30 lines per mm. for

a fast emulsion through an average of 60 up to as many as 1,000 for an emulsion of maximum resolution.[1]

These figures are based on the maximum number of lines per mm. which can be observed on the photograph of a suitable test-object of wholly black and white lines, giving maximum contrast, and of equal width.

The choice of suitable photographic material does not depend on grain alone, and we must assume that in the majority of cases emulsions of average resolving power will be used. The detail to be resolved, moreover, will rarely or never present optimum contrast, so that for this and other reasons connected with the complexity of the phenomenon, it is reasonable to adopt a safety factor of 10 ×.

Taking an average resolving power of 60 lines to the mm., and a safety factor of 10, we find that the smallest interval resolved by the objective must be so magnified as to cover 160μ on the surface of the emulsion.

When focusing on the camera screen a magnifier is invariably used for fine detail, and in the case of eyepiece cameras, the viewing or focusing eyepiece when provided will also magnify the image considerably, so that detail may be visible to the eye which will not be resolved by the emulsion. This is a point well worth bearing in mind.

Clearly the minimum only applies when the *full* resolving power of the objective is required.

Minimum magnifications (for emulsions of average resolving power) are given in the following table for standard objectives in general use:

Objective mm.	NA	Interval resolved. ($\lambda = 0{\cdot}55$)	Minimum magnification to cover 160μ on the plate. (Resolving power of emulsion 16μ)
16	0·25	1·1	150x
	0·30	0·9	200x
8	0·50	0·55	300x
	0·65	0·40	400x
4	0·85	0·32	500x
	0·95	0·30	550x
2	1·25	0·22	700x
	1·40	0·20	800x

[1] H. Baines, Proc. Ass. for Scientific Photography, February 1945.

This is seen to be very nearly 600x the value of the numerical aperture.

These figures will be safe whatever the emulsion used and this was the purpose of the foregoing notes. With special fine grain emulsions, relevant data should be obtained from the manufacturers, but there is no particular advantage in reducing these values to any great extent.

Any form of magnification before the negative stage will, of course, reduce both the brilliancy of the image and the area of the image contained within the plate.

Brilliancy is important for ease of observation when making adjustments, and also in order not to prolong exposure time unduly.

The extent of the object that fits within the plate is an important factor in order that the photograph may be intelligible, particularly in the case of small cameras.

These points have to be considered when deciding how to obtain the overall magnification finally required. This is best shown as follows:

(1) Initial objective magnification.	Range 5–120	This will depend on the *NA* required to resolve the finest detail present. It should be kept as low as compatible with this condition if maximum depth of focus is required. It should be as high as possible if "optical sectioning" is required.
→	First stage of Resolution.	←
(2) Eyepiece magnification.	Range 1–10	This should be considered in conjunction with (3). It should be kept low, for optical reasons.
(3) Camera extension.	Range 1–3	This will depend on (2). Long extensions are inconvenient, lead to tremor, and loss of brilliancy. Faint images may be very difficult to focus. It need rarely exceed 20 inches (2x).

Second stage of Resolution.

The total magnification at this stage should generally be of the order of $600x\,NA$. If less, the emulsion may not resolve finest detail. If very much more, the image will be faint and difficult to focus, the field of view small. Tremor may arise and adjustments are difficult.

(4) Photographic enlargement.	Range 1–4 (Limit is sufficient in practice for all purposes).	This will depend on the final magnification required. It is limited by the quality of the finished negative and, ultimately, by the grain.

Special Case of Eyepiece Cameras.—It should be noted that when using eyepiece cameras the projected image is usually *smaller* than the visual image because the distance from eyelens to screen is less than 10 inches.

With the box type camera, it may be of the order of 5 inches, and with a miniature camera, such as the Leica or Contax, of the order of 3 inches, unless some form of extension tube is used.

It follows that the objective x eyepiece magnification will be correspondingly reduced to a half or a third of its normal value.

With a 4-mm. objective, having an initial magnification of 40 diameters and an 8x eyepiece, the magnification on the screen of a 5-inch camera will be $40 \times 8 \times \frac{1}{2} = 160$ diameters.

A 4-mm. lens of the achromatic type, having a numerical aperture of 0·85, will resolve 0·32μ. This interval will be magnified to $160 \times 0{\cdot}32 = 51{\cdot}2\mu$ on the screen.

This is quite insufficient for focusing unless a focusing lens magnifying at least 4 diameters is used, and it may be quite insufficient for resolution by the emulsion used.

It is thus important to use material of the finest possible grain with such cameras and ultra-fine grain developers. This is all the more important because considerable photographic enlargement from the negative will be required.

Conclusions.—The resolution of the emulsion depends on grain, correct exposure, type of developer, correct development, quality of the projected image, and magnification.

Owing to the difficulty of satisfying these conditions fully and

simultaneously, it is advisable for general work to keep within the limits indicated, but these limits merely constitute a safeguard.

Long camera extensions, whether vertical or horizontal, are extremely inconvenient. It soon becomes impossible to observe the screen image and manipulate adjustments simultaneously. For this reason it is better to rely on the eyepieces for altering the magnification and to keep the camera extension down to 15, or at the most 20 inches.

The camera extension is very useful for:

(*a*) Fitting the image to the size of plate.
(*b*) Obtaining a standard "round figure" magnification.

It will be found that in practice these become the main functions of the camera extension, and that abnormally long extensions are of no advantage. The brilliancy of the image rapidly decreases as the camera is extended. The exposure problem is thus much simplified if a standard camera length is adopted, which is only varied within such limits as may be required to adjust the size of the field or round off the value of the overall magnification.

Optical Sections.—So far we have assumed that as much depth of focus as possible is required, and this is generally the case. But the problem can often be solved satisfactorily by passing to the other extreme. When photographing certain very fine structures it is often possible, by using a lens of high numerical aperture and considerable magnification, so to reduce the depth of focus that every detail lying on either side of a thin "optical" plane is so completely out of focus as to be entirely invisible on the photograph.

SUMMARY

The above points have been treated at some length because they are often a source of perplexity to beginners, and the relevant information is very scattered and often misleading, or even contradictory.

The whole matter can be summed up in the following few, simple, recommendations (rather than rules).

(1) Work for a magnification, on the screen of approximately 600 times the numerical aperture of the objective, as a minimum, and 1000 times as a maximum. Keep *NA* low for depth of focus.

(2) Keep the camera extension between 15 and 20 inches, using an eyepiece of correspondingly lower power.

(3) Use the camera extension between these limits to fit the image to the size of the plate, and to round off the magnification to a convenient whole number.

(4) If further magnification is required, enlarge from the negative.

Flatness of Field.—This is relatively unimportant for visual observation, but it cannot be ignored in photographic work. A high-power objective or, more properly, an objective of high numerical aperture, cannot produce an image of a flat object which is simultaneously in critical focus over the whole field. The field curvature becomes more pronounced as the numerical aperture is increased. Field curvature can, to a limited extent, be corrected by the use of special eyepieces, in particular the so-called "amplifying lenses" which are now available for projection and photographic work.

Generally speaking it is sufficient to bring the central portion of the field into sharp focus and block out the marginal area so as to obtain a sharp edge. This may be done by interposing a diaphragm of suitable size immediately in front of the plate, but it is simpler to do it in the usual way by screening when printing or enlarging from the negative (masks).

Can one dispense with an Eyepiece altogether?—Unless the magnification required is very low, and the structure very coarse, it is always good practice to use an eyepiece.

In the first place, an objective used without an eyepiece is forming an image far beyond the proper conjugate focus for which it is corrected. This difficulty may be overcome by removing the bodytube altogether, so that the camera screen can be brought up to the position where the eyepiece diaphragm would normally lie. This also does away with flare from the inner surface of the tube, but suitable light-proof screening must be provided for the sensitive plate and the image will be very small indeed. If a bodytube is used, it must be lined with black velvet.

In the second place, apochromatic objectives are corrected for use with compensating eyepieces and are not suitable for photographic work unless they are so used.

The use of an eyepiece lessens the camera extension required for higher magnifications, which is a great convenience in practice, and prevents much of the danger of stray light or flare reaching the plate from the tube, mainly owing to the presence of the field lens and eyepiece diaphragm.

Illumination.—Illumination is of the greatest importance. The principles explained in Chapter VI apply unchanged, with the following qualifications:

(1) Even, or uniform, illumination over the whole field is more important in photographic than visual work. The negative will show up such unevenness where the eye will fail to notice it.

(2) The illuminant should be powerful or the exposure times will be unduly prolonged, and the screen image at high magnification too faint for satisfactory focusing.

(3) Alignment is very important and, with long camera extensions, the distance from light-source to substage illuminator may be critical.

(4) The Köhler method, as previously described, should be used. It is the most generally satisfactory, particularly as regards evenness of illumination.

(5) Successful results depend on the proper use of filters in combination with the correct type of photographic material.

Apart from these recommendations, there is nothing to add to the methods earlier described.

When making preliminary adjustments by visual observation, the light must be reduced, or the eye may suffer damage, or a serious temporary loss of sensitivity.

Exposure.—We have seen that, although modern photographic emulsions give considerable latitude in this respect, the exposure must be kept down to the minimum consistent with a good negative, if maximum resolution is required.

Exposure is a matter of experience, just as it is in ordinary photography, and no more difficult to acquire. The expert photographer does not hesitate about exposure and needs neither tables nor formulae. The same is true of the experienced photomicrographer. *All so-called exposure formulae are far too complicated to be of the slightest use in practice.*

It is as well to realise upon what factors the time of exposure will depend.

The normal time of exposure varies as follows:

Inversely as the square of the numerical aperture of the objective and condenser.

Directly as the square of the magnification.

Directly as the filter factor for the light and plate used.

Inversely as the speed of the plate.

Inversely as the actinic intensity of the light source.

Exposure time can be tested on very small plates (having the same characteristics as the plates used) held within the dark-slide by means of an adapter.

Alternatively, the dark-slide cover can be raised so as to expose say, $\frac{1}{6}$ of the plate, and an exposure of 1 second (or any other value) given. The slide cover is now withdrawn so as to expose $\frac{2}{6}$ of the plate, and a further exposure of 1 second given, and so on.

The plate is thus divided into six (or any other number) zones which have received exposures of 1, 2, 3, 4, 5, and 6 seconds respectively. If the unit exposure is taken as 5 seconds, the values would be 5, 10, 15, 20, 25, and 30 seconds, etc.

Familiarity with the equipment, and the type of work done, will soon overcome such difficulties.

It is of great assistance to choose one particular type of emulsion, generally suitable for the work contemplated, and to stick to it.

Focusing.—Some cameras (particularly the eyepiece type using film) are provided with a focusing eyepiece which can be so adjusted that the virtual image seen through the eyepiece is in sharp focus when the real image projected into the camera is itself sharply focused in the plane of the sensitive emulsion.

This is a great convenience because the object can be observed right up to the moment when the photograph is taken. For photographing living specimens, some arrangement of this kind must be used.

Generally, however, the image is carefully focused on a ground glass screen, and this is not particularly easy. Sharp focusing is the first condition of successful photography.

It is not always realised that the exact position of the screen or plate is unimportant. Elaborate precautions are often taken (and described at length) to ensure that the plane of the sensitive emulsion exactly coincides with the plane of the glass or screen on which the image has been focused. This is quite unnecessary. The reason for this is that the diameter of the beam leaving the eyepiece is very small, usually of the order of 1 mm. The angle which the marginal rays make with the optical axis is therefore very small, of the order of 45 seconds of arc. Thus the focal range *in the image space* is a value of considerable magnitude.

For a camera length of 10 inches, the range is approximately 5 inches, so that there is an allowable movement of the plate of some $2\frac{1}{2}$ inches on either side of the position of the sharpest focus. The latitude increases with camera length. The magnification,

however, will vary as the plate is moved. It is best, of course, to work for complete coincidence in spite of this, but it is not essential. At low magnifications, the image can be focused on the screen (which should be of the finest possible grain) in the usual way without any difficulty.

At high magnifications it will be found that the image is very faint, and that the grain of the screen makes it impossible to

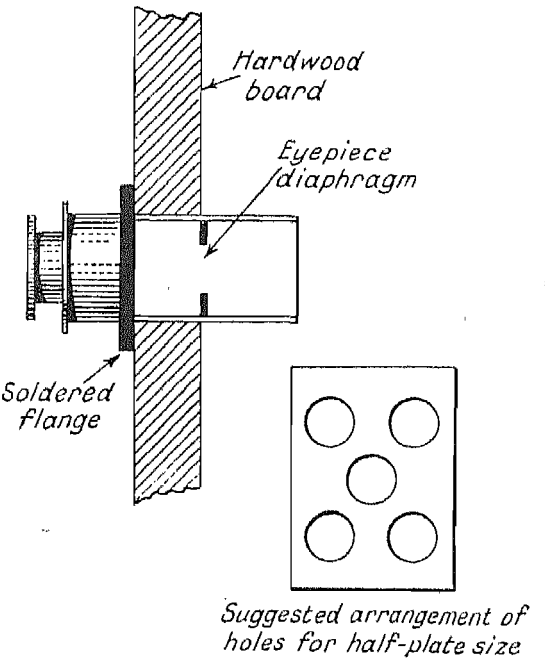


FIG. 41.

secure sharp focus. A plain glass screen should be substituted; the image will now be invisible, but it can be observed through a focusing lens, the effect of which is to substitute a virtual image which is visible to the eye. The focusing lens should of course be focused on to the underside of the glass, where the image is formed. It really acts as an eyepiece, and one of the best methods of focusing at high powers is to use a second eyepiece and to dispense with the glass screen altogether. This is best done as follows (Fig. 41):

A plain piece of $\frac{1}{4}$-inch hard wood is cut to fit into the back of the camera instead of the focusing screen. One or more holes

are cut in the wood with an adjustable bit and brace so that a low-power eyepiece can be pushed through, the plane of the eyepiece diaphragm being level with the inner face of the board.[1] This can be easily arranged by purchasing an old-type second-hand eyepiece and soldering a flange on the barrel which will come to rest against the outer surface of the board when the diaphragm is level with the inner surface.

On looking through the eyepiece, precise focusing with the highest powers and faintest images can be carried out just as easily as for ordinary visual observation.

The field of view is very restricted, so that only part of the image can be seen from any one position of the eyepiece. If the plate is small, one hole may be sufficient, but for a quarter-plate two, or even three holes, will be required. For half-plate size, a good arrangement is to have one hole in the middle and one in each corner.

The method does away with the necessity for adjusting the focusing lens, and leaves both hands free. (When the camera is used in a horizontal position, the usual focusing lens must be held against the glass.)

Instead of using a plain glass screen, a cover-slip, or several, can be cemented with balsam to the inner (ground) face of the ground glass screen which will then appear clear over those small areas. The eyepiece method just described will be found the best for really difficult work, but the eyepiece used should be of as low power as possible, particularly if the field lens is removed.

Within a certain range, it is frequently difficult to decide what is the correct position of focus. The entire aspect of the specimen may be altered, at high magnification, by a slight movement of the fine adjustment without any apparent change in sharpness. Structures near the limit of resolution are particularly subject to such changes, both in size and colour (they may change from black to white, or vice versa, and still remain sharp). With oblique or dark-field illumination, shadows or diffraction patterns may give spurious images.

Before attempting to take a photograph, therefore, the microscopist should familiarise himself thoroughly with the structure

[1] Owing to the latitude previously mentioned, the exact position is not important, nor does the presence of the field lens make any appreciable difference. If the field lens is removed, the image will be larger, and the field of view smaller. This is perhaps the better method for very critical images; and, the field lens being removed, the diaphragm should be level with the inner face of the board, this being so adjusted as to lie in the plane of the emulsion.

of the specimen by prolonged visual study under varying conditions of illumination and magnification.

Choice of Field.—This requires considerable experience and judgment. The field selected as representative must be truly so if the photograph is to be of value as a record for future reference. It must either be large enough to include all significant features or it must be restricted to one important feature for emphasis. In the latter case it is usually good practice to take a second photograph with a wider field in order to show the relationship of the part to the whole.

The size of the field depends on the magnification, so that here again the microscopist is faced with the necessity for compromise. Since an *intelligible* photograph is the ultimate aim, size of field and depth of focus (or optical sectioning) are just as important, in photography, as the degree of resolution. This fact is too often ignored.

Visual observation gives a much more vivid representation of the specimen. The observer can change from one objective to another, alter the plane of focus, or the field of view. A careful drawing can integrate various appearances, emphasise important details and suppress the unimportant. The photograph can do none of these things. Its value as a record depends on its freedom from the personal equation, but to be of any real use it must be so taken that its inherent limitations are overcome by careful preliminary study and a skilful combination of the means available to the photographer. This is no easy matter, and it is an unfortunate and misleading fact that the statement is so often found that photomicrography is nothing more than coupling a camera to the eyepiece and exposing a plate. That is a mere detail. Any photographer knows that composition is the secret of good photography. This is even truer in the case of photomicrography, but composition must have intelligibility as its primary aim.

II. APPARATUS

Standard apparatus, as supplied by the manufacturers, is abundantly described in their catalogues, and such information need not be duplicated here. I propose to deal with typical arrangements, such as can be set up with ordinary apparatus, and are suitable for work of a general nature, even of an exacting kind.

We have already seen that such apparatus belongs to either one of two main groups:

A. The eyepiece type.
B. The optical bench type.

Each of these will be dealt with in turn.

A. **The "Eyepiece" Camera Group.**—We are dealing here with light cameras of small size (and almost always of fixed length) which can be slipped over the eyepiece and clamped to the body-tube when it is required to take a photograph.

The advantages are convenience, rapidity, simplicity, and compactness. The disadvantages are size restrictions (particularly as regards magnification), though with the introduction of very fine grain emulsions, the range of action of this type of camera has been considerably extended.

Most eyepiece cameras use plates, but some use film. In the latter case provision must be made for focusing, either by:

(*a*) An observation eyepiece.
(*b*) A reflex focusing attachment.
(*c*) The twin camera method.

Most makers' cameras are designed for use with an observation eyepiece. A shutter and removable semi-transparent reflector are provided, and the reflector diverts about 25 per cent of the light into the observation eyepiece which is usually set at right angles to the optical axis of camera and instrument. By looking through this auxiliary eyepiece, the microscope can be focused while the plate is in position, and during the exposure if necessary. An adjustment provides for coincidence of visual and photographic foci.

The reflex attachment is used with certain types of "miniature" cameras. The principle is well known, but since the main purpose of the eyepiece camera is to "snap" phases in a process, or certain appearances in changing, or even living, material, any device embodying a ground glass screen is inconvenient for use with any but the lowest magnifications, and the observation eyepiece is far superior in this respect.

The "twin camera" method suffers from the same defect, but it has the advantage of great simplicity and low cost, and can be used for every purpose except instantaneous photography of living, or rapidly changing material.

Two identical box-type cameras are used. Any of the standard

models can be selected, but they must be as light as possible. They can also be cheap, because the lenses are not used.

First remove the lenses, then fit to the front of each camera a fairly deep wooden flange of sufficient internal diameter to slip easily over the eyepiece. The microscope being in a vertical position, these light cameras will "sit" quite securely in position, just resting on the eyepiece (Figs. 42 and 43).

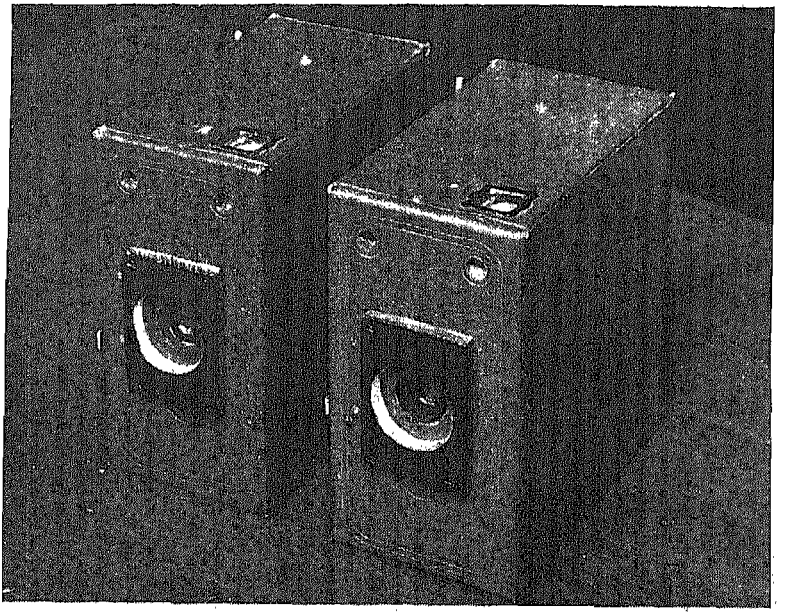


FIG. 42.

One of the cameras is loaded with film (or it can easily be adapted to take a small plate). The other camera has a ground glass screen permanently fitted over the film track, and a focusing hood can be added for convenience. This is easily made from an old folding camera bellows of suitable size.

The first is the true camera, the second is an exact focusing replica. The procedure is obvious. First place the focusing camera over the eyepiece and focus in the usual way. Then substitute the second camera and expose the film or plate.

The shutter is used *only* to make the camera light-proof when it is not in use. The exposure is made by interposing a black card between the mirror and substage illuminator *before* the camera

shutter is opened and withdrawing it to expose the film. At the end of the exposure, the card is again interposed, the shutter closed, and the camera removed. The reason for this is that the type of shutter fitted to these cameras, and the fact that the camera is merely resting on the top of the tube, would lead to tremor if the camera or shutter were touched at any time during the exposure. The room should be as dark as possible.

Such an arrangement is extremely convenient. The cameras are always ready for use, and no elaborate adjustments are

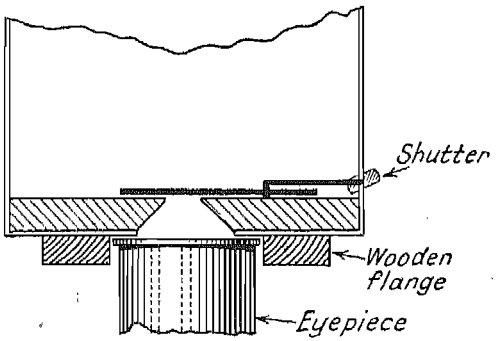


FIG. 43.—Arrangement of box-camera on eyepiece.

necessary. A third identical camera can be adapted to take small plates, for the purpose of testing exposure times, or where immediate development is required for any reason. My own focusing camera is fitted, instead of a ground glass plate, with a thin wooden slide through which a focusing eyepiece is fixed, in accordance with the method I have already described. The cameras are Ensign E.129, and are extremely light, being mainly made of cardboard.

Makers of miniature cameras, such as the Leica or Contax, supply special attachments and extension tubes for use with microscopes. Full details and literature can be obtained from the manufacturers.

Generally speaking it is not good practice to use a large eyepiece camera (some are made to take quarter- or even half-plate). It is better to use the optical bench type of equipment for negatives of any size, because the inevitable weight of such cameras may affect the microscope and its adjustments. If very small sizes are used (such as the 35-mm. miniatures), either the magnification

must be kept small or the field of view will be very restricted. Enlargement from the negative will almost always be necessary, but this does not get over the difficulty that initial magnification is linked with numerical aperture on which resolution alone depends.

The ordinary box camera has the advantage in this respect The size of the negative is usually $2\frac{1}{2}$ inches by $3\frac{1}{2}$ inches, and this is sufficient, without further enlargement, for many purposes.

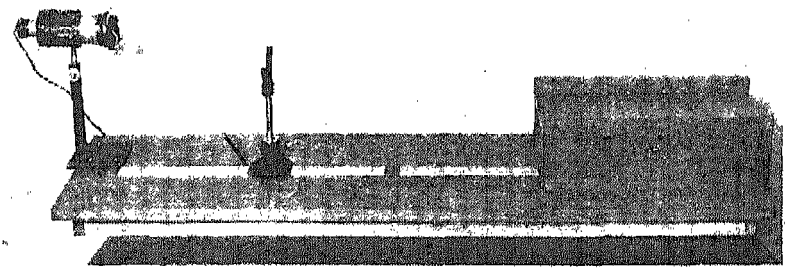


FIG. 44.

B. **The Optical Bench.**—The true optical bench really belongs to the third group of equipment mentioned at the beginning of this chapter, and is a very elaborate piece of apparatus only justified in very special circumstances and, as such, outside the scope of this book. The term is extended, however, to include any arrangement by means of which a camera of the studio or field type, a microscope, various optical devices and a light source, can be properly aligned, and maintained in alignment. This form of equipment is in general use, and is capable of fulfilling all the conditions required by work of the most exacting kind. This will, of course, depend primarily on the quality of the microscope and its optical components.

I do not propose to enter into a discussion of the relative merits of the vertical or horizontal types. For certain classes of work the microscope must be used vertically, and there can be no further argument. Otherwise, the horizontal type is more

convenient, particularly for long camera extensions (though these are quite unnecessary, as we have seen), and it is certainly sounder from a mechanical point of view.

A strong hard-wood base-board of ample size (at least 1 foot 6 inches by 5 feet) is required, and this should be strengthened by means of cross battens to prevent warping. At one end of the board a rigid platform should be built to raise the camera so that its optical axis is level with that of the microscope when the latter

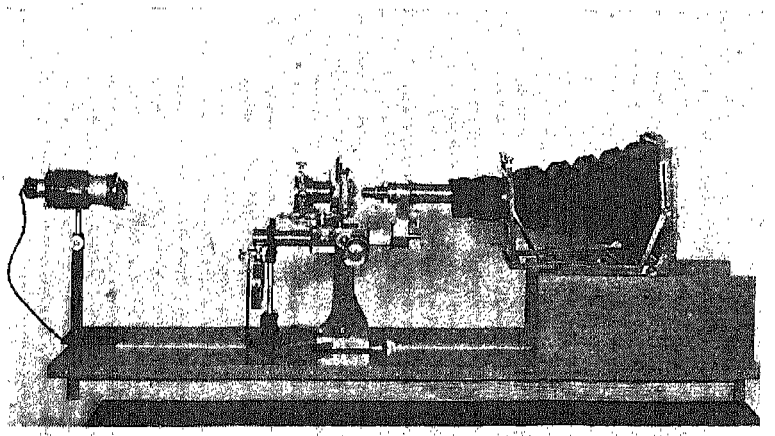


FIG. 45.

is in a horizontal position. This platform should be about as long as the camera when the latter is fully extended, and the camera should be arranged to slide along the platform between guides. This will be a great convenience in many cases. The camera can also be instantly removed, for visual adjustments, and accurately replaced in its guides, a graduated scale and pointer being provided. Any standard camera will do. It should extend to 25 inches, and both the back and front of the camera should be movable. Half-plate size is best. Adaptors can easily be made to hold smaller plates. The simplest and most effective form of light-tight connection is a sleeve of dark cloth fitted to the camera front, with an elastic band at the other end (sewn in) to slip over the bodytube. The sleeve should be short or it may sag and interfere with the field (Figs. 44, 45).

Alignment.—(1) The microscope is first set in a horizontal position accurately along the main axis of the board. Small brass

angle pieces can be screwed into the board so that the microscope feet register against them. This ensures that the instrument is always replaced in correct alignment without further adjustment. (See (6).)

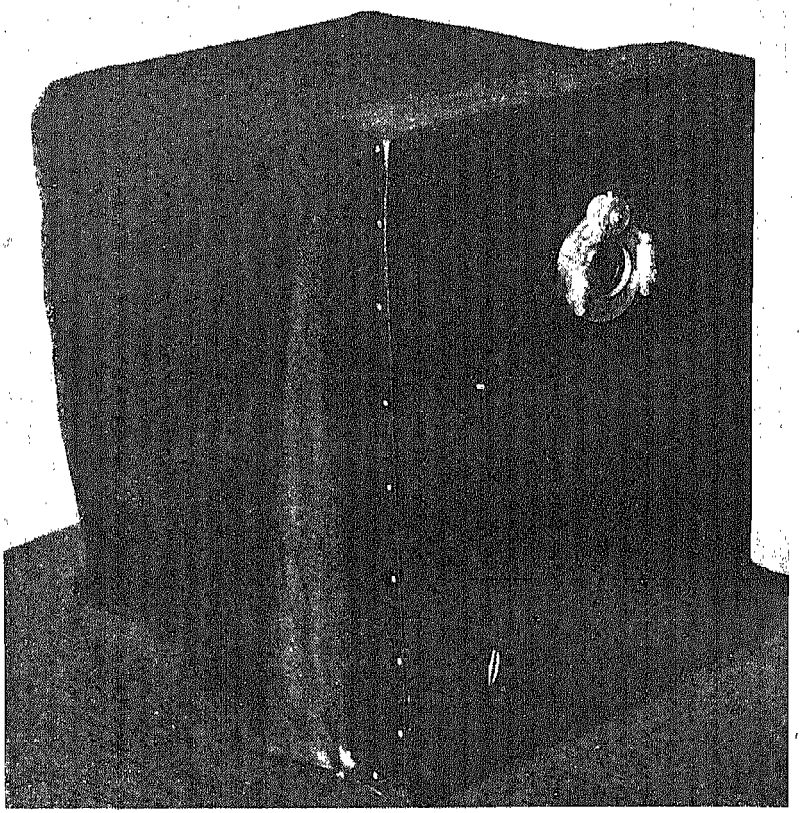


FIG. 46.

(2) It is now necessary to adjust the instrument so that its optical axis is truly horizontal. The easiest way to do this is by means of a tool maker's surface gauge. This consists of a pointer carried on a pillar and a heavy accurately machined base. The height of the tip of the pointer can be adjusted with great

precision, and clamped firmly in position. The microscope is fitted with a dummy pin-hole eyepiece, and the substage diaphragm (previously centred) is closed completely.

The surface-gauge pointer is now adjusted so that it is just level with the eyepiece pin-hole, and the gauge can now be moved along to test the corresponding level of the substage diaphragm pin-hole. In this way, the microscope can be set horizontally and once set, a metal crutch mounted on the board, preferably under the tail-piece, can be so adjusted as to hold the instrument in this position. A light coiled spring, hooked to the tail-piece just beyond the crutch, will hold the tail-piece down on to the crutch.

Once these adjustments have been made, the microscope can be removed and instantly replaced in exact alignment without further difficulty. All other components are aligned on the microscope axis.

(3) The surface-gauge, now adjusted to the correct level, can be used to check the vertical alignment of camera, condensing lens and light-source. It will be found that this is very convenient for preliminary adjustment.

(4) Finally, horizontal alignment and vertical alignment can be further checked by optical means. Exact centring of the light-source is not essential, but the condensing lens diaphragm can be closed down to a very small opening and, when focused on to the camera screen, it should be centred on the screen cross-diagonal intersection.

Any further adjustments are carried out with the optical components in the usual way. It may be that the centre of the field will not be exactly in the centre of the screen, but this is of no great importance and the displacement will be very small if the preliminary adjustments with the gauge have been carefully carried out.

(5) The condensing lens and light-source are best mounted on heavy metal bases sliding between guides. A good plan is to cut V-grooves on the under surface of the bases, and run them on cylindrical metal rails fixed to the base-board, but these rails need very careful alignment.

(6) The surface-gauge, used to check alignment in the vertical plane, can also be used to check alignment in the horizontal plane when setting up the bench. For this purpose a steel straight-edge is screwed on to the board, parallel to its main axis, and the machined face of the gauge base can be made to slide along this

edge. The tip of the pointer, set to coincide with the eyepiece pin-hole and substage diaphragm centre when the microscope is in correct alignment, will now move along a horizontal axis on which all other components can be accurately set.

The entire bench can be mounted on rubber sponge pads to eliminate vibration, and it is best to install such apparatus on a ground floor or in a basement, to avoid tremor, which is fatal when using high magnification.

For the same reason, it is inadvisable to use a shutter on the camera. On the other hand, the black card arrangement is a little crude.

A good method is to use a wooden screen (an ordinary wooden office letter tray is ideal) in which the shutter is mounted. The wooden screen is carried on an independent support resting on the table and bridging the base-board between the light source condensing lens and the substage condenser. The screen can be provided with a black cloth hood which can be drawn over the light-source. This is of great assistance when focusing faint images (Fig. 46).

A good Compur shutter with bulb or bowden release is ideal.

Choice of Photographic Material.—Here again, the manufacturers' pamphlets give the fullest information, and this is the only reliable guide in view of the fact that such material will invariably be used with filters, usually supplied by the same manufacturers. The following points, however, may be helpful.

The main thing to remember about the characteristic curve of a material is that it is *not* characteristic of the material.[1] It is characteristic of the combination: material, exposure, processing, drying, and densitometric conditions. Exposure includes the actinic characteristics of the light-source which have a profound effect on the behaviour of the material. Processing includes composition of developer, time and temperature of development, degree of agitation, etc.

Speed, contrast, grain, and colour sensitivity are the main factors. Lists of emulsions are usually arranged in order of speed, measured under normal working conditions, and sub-divided according to colour sensitivity.

In general, the image to be recorded is coloured, full of detail, and of relatively low contrast, so that the emulsion should be

[1] H. Baines, "The Choice of Material for Scientific Photography" and F. J. Tritton, Ass. for Scientific Photography, February 1945.

panchromatic, fine-grained, and of relatively high contrast. The most widely used plate is thus Process Panchromatic (P.150 Kodak). Such plates should not be developed in a high contrast developer and development time should be on the short side. For rather less contrast, Ilford Special Rapid Pan or Kodak P.500 are suitable.

For the photography of polished or etched metal surfaces, orthochromatic plates are best.

Blue-violet sensitive emulsions of the "Ordinary" type and process grade are excellent for use with objectives of very high resolving power and blue-violet filters.

It is better not to try too many alternatives, but to adopt one or two types of emulsion, say a process and a panchromatic, and become thoroughly familiar with their characteristics.

INFRA-RED PHOTOGRAPHY

Unlike ultra-violet photography which requires special lenses and expensive equipment, infra-red photography can be practised just as easily as ordinary photography. All that is required is a suitable filter and infra-red plates or films. The ordinary lamp is quite adequate as an illuminant. A great deal of additional detail is revealed particularly with entomological and other chitinous specimens.

Precautions should be taken to guard against the penetrating powers of these rays, and wooden plate holders should be avoided. The camera bellows and wooden body should be lined with special safety paper.

The main difficulty is focusing. The simplest method is given by F. D. Armitage (*The Microscope*, Vol. II, No. 5, p. 124), and was first suggested by H. Naumann. The fine adjustment should have a calibrated drum. The image is first focused visually through a green filter, and the fine focus calibration noted. The green filter is replaced by a deep red, and the new reading noted. To focus for the infra-red filter, the fine adjustment is turned in the same direction as when changing from green filter to red, but by exactly twice the amount. This is correct for apochromats, but the factor should be 1·4 instead of 2 for achromats.

Suitable filters for focusing are:

Green passing 5200–5800 Å
Red ,, 6200 Å.

CHAPTER XI

SPECIAL INSTRUMENTS AND APPLICATIONS

THE microscope has been applied to many fields, and some of these applications have become so important that the basic design of the instrument has been considerably modified in consequence.

These instruments cannot be studied without reference to the work for which they are intended, and the only adequate sources of information are thus the specialised text-books concerned.

The purpose of this chapter is merely to give the reader some indication of the nature of these instruments. It is an enumeration with a few brief comments. Needless to say, however unorthodox an instrument's design may be, the fundamental principles apply unaltered.

Modifications in design are only a matter of convenience, the suppression of the unessential, and the emphasis of that particular component or feature which is of greatest importance in any special field.

BINOCULAR INSTRUMENTS

The value of stereoscopic binocular vision with low powers is unquestioned, and the improvement in such devices has extended their usefulness to high-power work, but one must be careful to discriminate between what is stereoscopic vision, and what is not. Some forms of binocular instruments do not give stereoscopic vision, and are not intended to do so.

We may thus first divide binocular devices into two main groups.

(1) Devices for stereoscopic vision.

(2) Devices which present to the eyes two identical and congruent images (non-parallactic vision).

Instruments may be further sub-divided into three groups:

(*a*) Those which have two bodytubes and *two* objectives arranged at a convenient angle so that their focal points coincide in the object plane.

These are instruments of the familiar Greenough type, suitable only for low powers (a considerable working distance is a

mechanical necessity). They are much used for dissecting, for industrial applications, and particularly for tool-making. A great variety of designs is available, incorporating many special features, but the fundamental principle remains the same (Fig. 47).

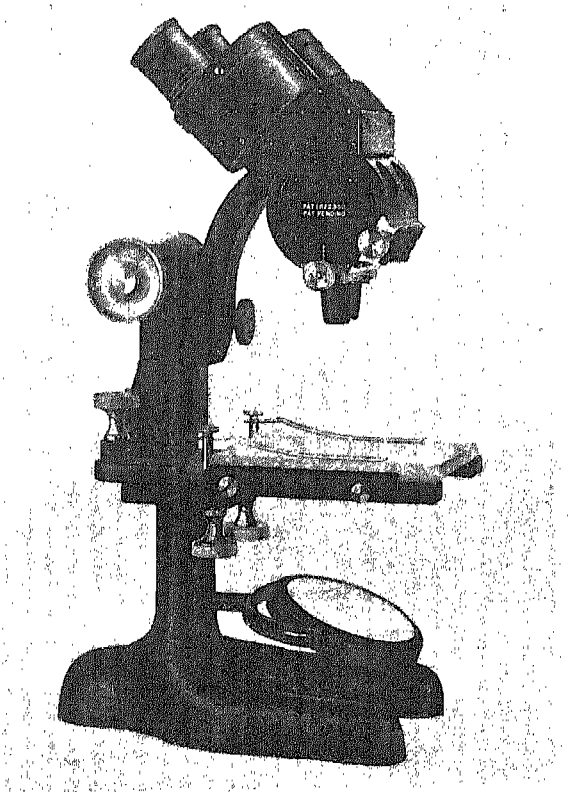


FIG. 47.—Typical Greenough binocular. (Bausch & Lomb)

They give true stereoscopic vision.

(*b*) Those which have two bodytubes and a single objective.

These are two varieties:

(1) The Wenham type (Fig. 48), in which the beam from the single objective is split, by means of a prism, immediately behind the back lens of the objective.

This gives true stereoscopic vision, but at the cost of halving

the aperture of the objective. Owing to the difficulty of placing the prism close enough to the back lens, the method is restricted to low powers, up to $\frac{2}{3}$ inch.

(2) The Powell and Lealand type (Fig. 49), later modified by Beck, in which the image presented to each eye is formed by the full beam from the objective.

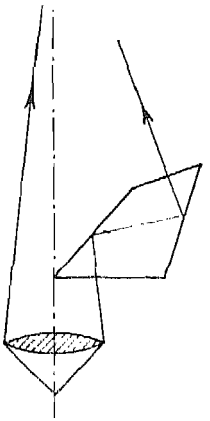


FIG. 48.—Wenham prism.

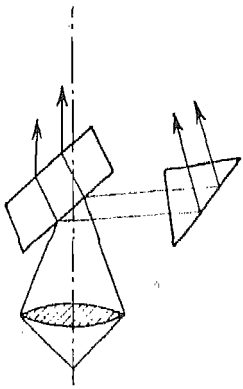


FIG. 49.—Powell and Lealand prism.

In all such cases the stereoscopic effect is lost and can only be restored by the use of special semicircular stops in the eyepiece.

The Wenham type is the best for true stereoscopic vision with low powers. It is unsurpassed in this respect.

(*c*) Those which have a single bodytube and objective, and a binocular eyepiece fitting.

This is really an extreme case of (*b*) (2) described above. Such attachments are mainly used for comfort and convenience when using high magnifications over long periods.

The binocular head fits into the monocular body in place of the ordinary eyepiece. It is possible to obtain stereoscopic vision by fitting stops in the plane of the exit pupil, but it must be borne in mind that stereoscopic vision means vision in depth, and that this has little or no meaning with objectives of high power and aperture, for use with which these attachments are mainly designed.

The image formed by such objectives is almost a true optical section, and this effect is often purposely achieved. Stereoscopic vision is meaningless in this connection.

The *raison d'être* of the modern binocular attachment is comfort and not stereoscopic vision.

MODIFIED STANDS

Certain types of stands have been profoundly modified to meet the requirements of workers in one particular field to the exclusion of others.

In such cases adaptability, or multiplicity of purpose, is useless and inconvenient.

There are many stands of this type, and the tendency to produce them increases, as the use of the microscope extends to embrace new and more specialised applications.

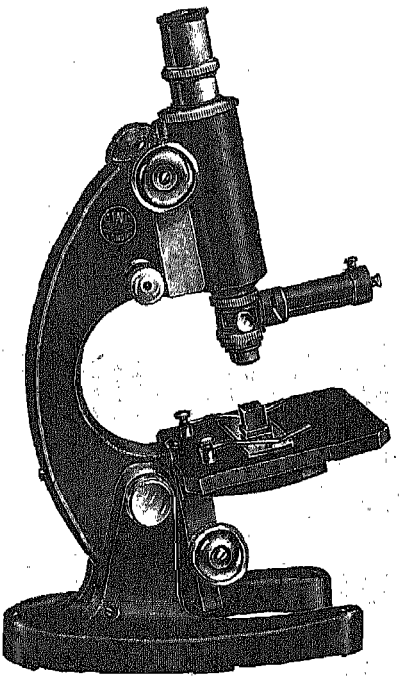


FIG. 50.—Typical metallurgical stand. (C. Baker)

The Metallurgical Microscope.—In the case of metals, illumination is always by reflected light. This fact controls the design of the metallurgical stand.

The substage fittings, illuminator, and mirror are no longer required, and the stage itself is solid.

It is usually made to move up and down by means of a rack motion partly for convenience in handling bulky specimens, and partly for adjusting the focus without interfering with the lighting arrangements.

Some form of vertical illuminator is generally incorporated. The stand is extra heavy, but otherwise very simple (Fig. 50).

The Mineralogical or Petrological Instrument.—There are two main factors which control design.

(*a*) The use of polarised light.

(*b*) The measurement of angles.

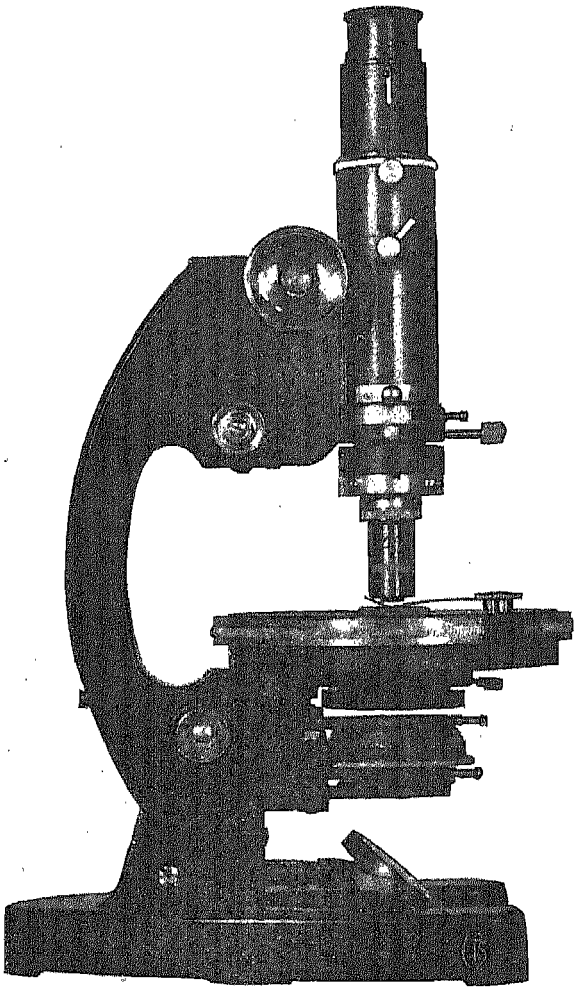


FIG. 51.—Typical petrological stand. (Cooke, Troughton & Simms)

It so happens that in the case of polarised light, angular measurement is equally important, so that the instrument is

primarily designed with this object in view. Illumination will be both for reflected and transmitted light, and ample provision is made for polariser fittings. These are provided with finely graduated scales.

The stage will be of the rotating type, graduated for angular measurement. Such instruments are also used for chemical work.

A slot is provided between objective and analyser for inserting compensators, and another between analyser and eyepiece to accommodate a special lens for observing interference figures. Provision may have to be made for water or electrically heated stages, and for the study of crystals synchronic rotation will be required.

These stands are more elaborate, and more highly specialised than any other type (Fig. 51).

For spectrographic work, spectrographic eyepiece attachments and spectrographic condensers are used. The former provide a spectrum of the light passing through the instrument, and there is an arrangement whereby this spectrum may be compared with a standard spectrum. The spectroscopic condenser superimposes a spectrum illumination on the object. Monochromators can be used as illuminators, providing a field of any spectrum region required.

RESEARCH INSTRUMENTS

The research instrument is the most perfect form of the general purposes microscope (Fig. 52).

It is of heavy construction, for stability and freedom from tremor. The stage is of large size, and can usually revolve. It can be accurately centred, and is graduated. The bodytube is of large diameter. Usually two interchangeable bodytubes are supplied, a binocular and a monocular.

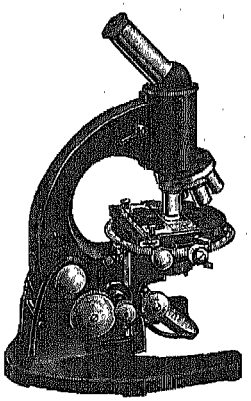


FIG. 52.—Research stand. (C. Baker)

During the last few years the reversed type (Fig. 53) has come into use. The supporting limb is on the far side. There is much to commend this design, but the stage cannot be used in an inclined position, though this is a very minor point.

The cost of these instruments is naturally very considerable, and they are profitable only when in the hands of an expert.

To use such an instrument for anything but the finest work is obviously inefficient.

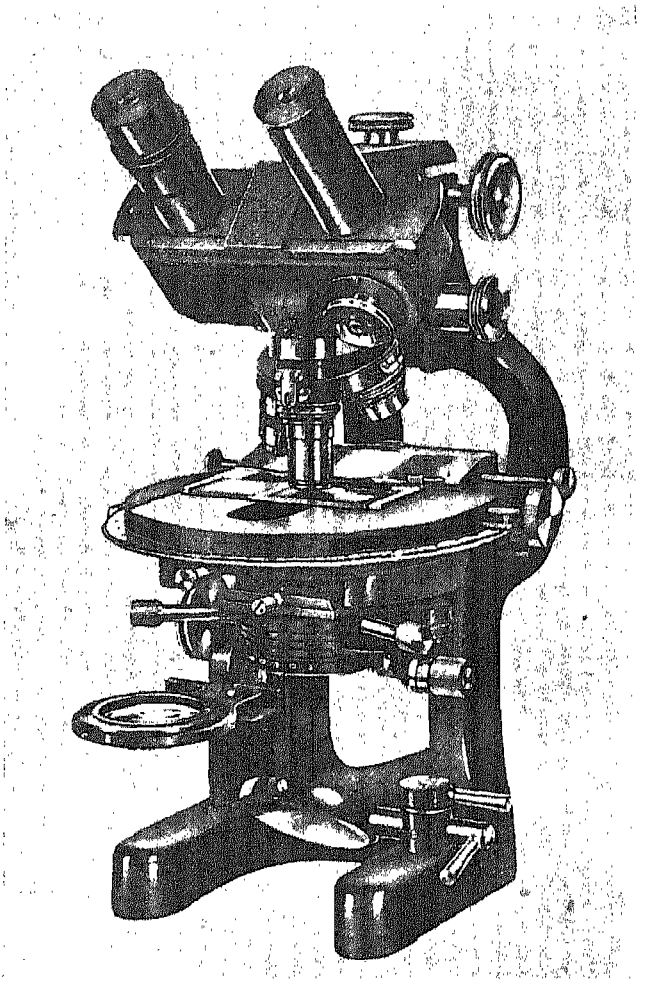


Fig. 53.—Typical research stand. (Bausch & Lomb)

POLARISED LIGHT

The use of polarised light has added very considerably to the usefulness of the microscope, so much so that, as we have just

seen, certain instruments are designed solely for use with this form of illumination.

Apart from its usefulness in geology, mineralogy, chemistry, etc., polarised light can be of great assistance to the metallurgist and to the biologist.

For this reason polarising attachments are standard components included in most general purposes equipments.

The principle is well known. Plane-polarised light means that the radiation is such that vibration directions perpendicular to that of propagation can be confined to one particular plane only.

If any two polarising devices are arranged in optical train and so oriented that the polarisation planes are parallel, light from one will pass through the other. If one of them is rotated so that the planes are perpendicular, no light will pass.

When certain objects are examined between polarisers, certain effects are produced which may:

(*a*) Reveal new structure by differentiation.
(*b*) Enable the observer to identify minute particles of crystals, minerals, organic materials, etc.

Both polariser and analyser, as the two polarising devices are called, are arranged to rotate about the microscope axis. The polariser is mounted in the substage, immediately in front of the condenser, and the analyser either just above the objective, or sometimes above the eyepiece.

The polariser and analyser are usually Nicol prisms, or calc-spar. For precise polariscopic determinations these have never been surpassed, but these prisms present certain disadvantages in some circumstances. A prism polariser which would utilise the entire aperture of a modern condenser would be prohibitive in price. The use of an analyser above the eyepiece has certain advantages, but a prism in this position is awkward and restricts the field of view.

Recently, it has been found possible to obtain large sheets of polarising crystals, all lying in one direction. These are known as Polaroid, and sheets or discs of these can be used instead of the usual Nicol prisms for some forms of work.

Polarised light can be of great assistance in general work. It can be used, under certain circumstances, to reduce glare.

Whenever contrast is poor, polarised light should be tried; typical examples are various vegetable fibres—cotton, linen, etc., which are almost invisible in balsam but brightly coloured under polarised light.

Manual dexterity, although subordinate to many higher mental qualifications, is as essential for the successful prosecution of microscopic observation, as it is for that of every kind of experimental science. BEALE.

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